Supplementary Data

Fabrication of injectable CuS nanocomposite hydrogel based on UCST-type polysaccharide for NIR-triggered chemo-photothermal therapy

Yueyuan Zheng,‡[a] Yuqing Liang,‡[a] Depan Zhang,[a] Zhijun Zhou,[b] Juan Li,[a] Xiaoyi Sun*[a] and You-Nian Liu[a]*

College of Chemistry and Chemical Engineering, Central South University, Changsha, Hunan Province 410083, P.R. China.
Department of Laboratory Animals, Central South University, Changsha, Hunan Province 410083, P.R. China.

Corresponding authors. Fax: +86-731-88879616; Tel.: +86-731-88879616. E-mail: juanli@csu.edu.cn (J. Li), xiaoyisun@csu.edu.cn (X. Sun)

‡These two authors contributed equally to this work.

Experimental Section

Materials.

Gellan gum (used for plant tissue culture) was purchased from Wako Pure Chemical Industries, Ltd. (Japan). Copper(II) chloride ($CuCl_2 \cdot 2H_2O$) was obtained from Tianjin HengXing Chemical Reagent Co., Ltd. (China). Sodium sulfide ($Na_2S \cdot 9H_2O$) was obtained from Tianjin Fengchuan Chemical Reagent Technologies Co., Ltd. (China). Sodium citrate was obtained from Henan Jiaozuo Chemical Reagent Co., Ltd. (China). Doxorubicin hydrochloride (DOX, 98%) was obtained from Huafeng Co., Ltd. (Beijing, China). Deionized water was obtained from a Synergy® Water Purification System.

4T1 cells obtained from Xiangya Hospital Central South University (Changsha, China) were cultivated using high-glucose DMEM (Hyclone) containing 10% fetal bovine serum (Hyclone) and antibiotics (100 units/mL penicillin and 100 μg/mL streptomycin), and were incubated at 37 ^{o}C in a humidified atmosphere containing 5% CO_2.

One-pot synthesis of nanocomposite hydrogel.

Into 100 mL of an aqueous solution containing gellan gum (2%), $CuCl_2 \cdot 2H_2O$ (1.27 mM) and sodium citrate (0.02 g) was added under stirring at room temperature. The pale-blue mixture solution turned dark-brown immediately upon the addition of $Na_2S \cdot 9H_2O$ (1.46 mM). Then, the reaction mixture was heated to 90 ^{o}C and stirred for 15 min until a dark-green solution was obtained. The nanocomposite (NC) hydrogel was formed after cooling at room temperature, and stored at 4 °C.

To incorporate DOX into the NC hydrogel, DOX was pre-loaded into the above mixture solution after CuS formation prior to gelling (around 50 °C), and further cooled down at room temperature. The final DOX concentration is 1.0 mg/mL.

Synthesis of citrate-coated CuS nanoparticles.

Citrate-coated CuS nanoparticles were prepared similar to the method by Zhou et al.[1] Briefly, $CuCl_2 \cdot 2H_2O$ (0.07 g) and sodium citrate (0.02 g), $Na_2S \cdot 9H_2O$ (0.10 g) were first dissolved in 400 mL deionized water under stirring at room temperature. Then, the reaction mixture was heated to 90 ^oC for 15 min. The obtained dark-green solution of citrate-coated CuS nanoparticles was cooled to roome temperature and stored at 4 °C.

TEM and SEM measurement.

Twenty μL of NC hydrogel solution after dilution was dropped onto copper net for TEM (FEI Titan G2 60-300 with spherical aberration correction, USA) characterization.

SEM characterization was performed on a scanning electron microscope (Nova NanoSEM 230, FEI). One droplet of hydrogel solution was dropped onto a silicon slice, and then the hydrogel samples were freezed at -20 ^oC for 12 h, then the samples were lyophilized, and sputter coated of a thin gold layer.

Optical properties, photothermal effect and photostability of NC hydrogel.

The NIR optical properties and stability of NC hydrogel were studied using SHIMADZU UV-2450. NC hydrogel was diluted by deionized water to a series concentration after melting at 70 °C.

To investigate its photothermal effect, NC hydrogel (about 1.0 g) were exposed to a fiber-coupled continuous semiconductor diode laser (808 nm, Beijing Viasho Technology Co., Ltd., China) at the power of 1.0 W/cm^2. The temperature of the NC hydrogel was recorded with an infrared thermal camera (Flir C2, USA).

To explore the photothermal stability of NC hydrogel, the NC hydrogel was illuminated by the 808 nm NIR irradiation at the power of 1.0 W/cm^2 for 3.0 min, and then naturally cooled to around 30 ^{o}C, the cycle was performed 5 times and the temperature was recorded by the infrared thermal camera.

Calculation of the photothermal conversion efficiency.

The 808 nm laser heat conversion efficiency (η) can be determined using the following equations.[2]

$$\eta = \frac{hS(T_{max} - T_{max,\,control})}{I(1 - 10^{-A_{808}})} \quad (1)$$

$$hS = \sum mC_p/\tau_S \quad (2)$$

$$\tau_S = -t/\ln\theta \quad (3)$$

$$\theta = (T_{surr} - T)/(T_{surr} - T_{max}) \quad (4)$$

where h is the heat transfer coefficient, S is the surface area of the container, container. τ_S is the sample system time constant, m is the mass of products (≈ 1.0 g), C_p is specific heat capacity of water (C_p = 4.2 J/mol), and the value of τ_S is 333.3 s obtained from Fig. S2.

T_{surr} is the temperature of the surroundings, T_{max} and $T_{max,control}$ are the equilibrium temperature of NC hydrogel and water, respectively. The deviation of T_{max} and $T_{max,control}$ is 40.9 ^{o}C. I is the laser power density (1.0 W/cm^2), and A is the absorbance

of NC hydrogel at 808 nm (A_{808} = 1.254). The photothermal conversion efficiency η is 54.6%.

Rheological measurements.

Rheological properties of NC hydrogel were performed on a rheometer (Anton Paar, MCR 302) with parallel plate geometry (25 mm in diameter). During measurement, silicon oil was applied to seal the parallel plate in case of water evaporation. The gap was set at 1.0 mm, and the frequency was set at 1.0 Hz for the rheological experiments. Storage modulus (G') and loss modulus (G'') was measured. For the temperature sweep experiment, the cooling/heating rate was set at 1.0 ^{o}C min^{-1}. To measure the sol-to-gel transition temperature, the NC hydrogel was first melted at 80 ^{o}C, and then about 1.0 mL solution was quickly added onto the plate with a settled temperature of 70 ^{o}C to avoid the gelling. Afterwards, the temperature sweep was conducted at a constant frequency of 1 Hz and strain of 1.0% automated controlled by the machine.

The gelation kinetics at 37 ^{o}C experiments was conducted at a constant frequency of 1 Hz and strain of 1.0 %. The samples were melted at 80 ^{o}C and then about 1.0 mL solution was added onto the plate which was settled at 70 ^{o}C. A program of temperature equilibrium at 37 ^{o}C was first run, and then the time-sweep measurement was started.

For continuous step-strain measurements, strains of 0.1% for 180 s and large strains of 300% for 30 s were alternatively applied. High-magnitude strain (300%) was applied to damage the hydrogel network for 30 s; afterward, low-magnitude strain

(0.1%) within the linear viscoelastic range was applied to monitor the recovery of the hydrogel structure.

In vitro photothermal therapy.

4T1 mouse breast cancer cells were seeded into a 6-well plate at density of 2×10^5 cells per well. After overnight incubation, the cells were adhered, the medium was replaced by fresh DMEM medium, and then one piece of NC hydrogel (~ 0.1 g) was placed onto the surface of cells. The cells were irradiated under an 808 nm laser at 1.0 W/cm^2 for 3 min. Subsequently, the hydrogels were removed and the cells were cleaned by culture medium. The cells were further incubated for 30 min and then stained with DMEM medium containing propidium iodide (PI) and calcein-AM for 30 min. Calcein-AM and PI differentiate the live and dead cells, respectively. Finally, the cells were washed twice with saline and imaged using an inverted fluorescence microscope (I3-TPC, Olympus, Japan).

In vitro NIR-triggered release.

The DOX-loaded NC hydrogel (1.0 g, containing 1.0 mg of DOX) was added with 2.0 mL of PBS buffer (pH 6.8, 0.10 M), followed by incubation in a water bath at 37 °C to mimic the tumor microenvironment.[3] For the NIR-triggering group, the NC hydrogel was irradiated with a 808 nm NIR laser within an interval of 30 min. The temperature of the hydrogel was elevated to 50 ^{o}C by NIR and kept for 5 min. During the irradiation, the temperature is monitored by thermo camera and finely tuned by the dose of irradiation. After the laser irradiation , the hydrogel was put back to 37 ^{o}C water bath, an aliquot of release medium (1.0 mL) was collected in every 30 min for

quantification and replenished with fresh release medium. The concentration of released drugs was measured from the UV-Vis absorbance at 496 nm. DOX-loaded hydrogels without irradiation were used as comparison. The stability of DOX in the hydrogels upon NIR irradiation was checked by fluorescence assay. The released DOX exhibits the same emission spectra without new bands as the free DOX (Figure S5B), indicating the DOX in the hydrogel is insensitive against the NIR irradiation employed.

In vivo photothermal therapy.

The 4T1 cells (1×10^6 cells in 100 μL PBS) were subcutaneously injected into the right back of the Balb/c mice. When the tumor size reached above 50 mm^3, the mice were divided into four groups (n = 4 per group): (a) PBS; (b) DOX; (c) NC hydrogel + NIR; (d) DOX-loaded NC hydrogel + NIR. The tumor-bearing mice were intratumoral injected with 200 μL PBS, DOX, NC hydrogel and DOX-loaded NC hydrogel. The dose of DOX was 10 mg/kg. In the case of the NIR treatment group, the tumor-bearing mice were anesthetized by sodium pentobarbital and exposed to NIR light (808 nm, 0.3 W cm^{-2}) for 3 min. The NIR irradiation was carried out 2.0 h after the injection. The tumor sizes were measured by a caliper on the 4^{th}, 8^{th}, and 16^{th} day after the treatment and no mice died during the course of therapy. The tumor volumes were calculated by the formula, tumor volume = $width^2 \times length \times 0.52$. On the 16^{th} day after treatment, the mice was euthanized, the collected tumors and organs (heart, liver, spleen, lung and kidney) was stained by H&E.

To assess in vivo repeated photothermal effect, the tumor-bearing mice was

injected either by NC hydrogel or citrate-coated CuS nanoparticles using the same procedure as described above. During each NIR irradiation, mice were anesthetized and then exposed to the NIR laser (0.3 W/cm^2) for 3 min. This process was repeated every day and a total of three sessions were recorded in temperatures using an infrared thermal camera.

All animal experiments were performed according to the ethical guidelines, principles, and protocols for the care and use of laboratory animals by Xiangya Laboratory Animal Center of Central South University.

Supplemental figures.

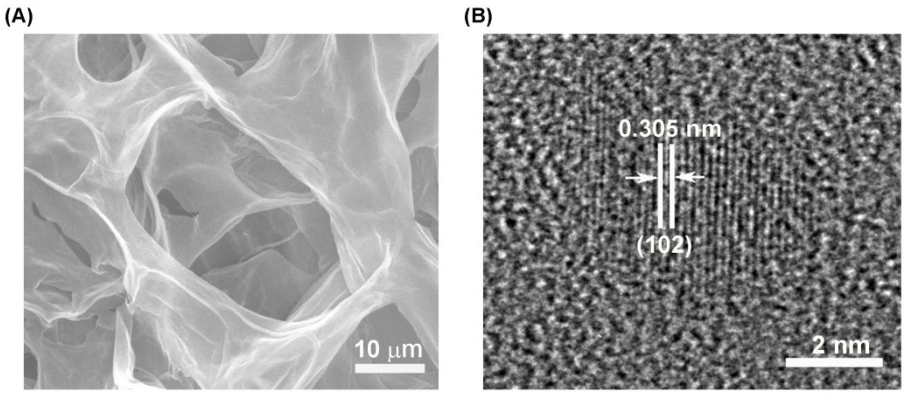

Fig. S1. (A) SEM image and (B) high resolution TEM image of the NC hydrogel.

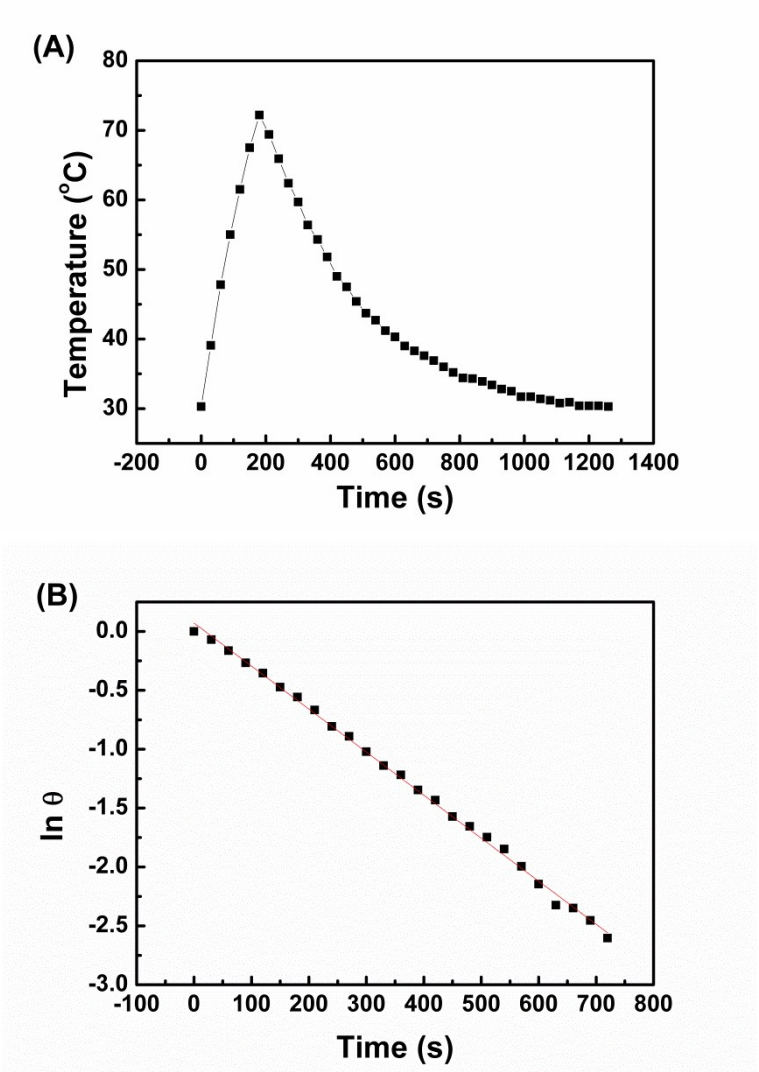

Fig. S2. (A) Temperature variation of NC hydrogel with laser irradiation for 180 s followed by natural cooling with laser off. (B) Calculation of the time constant for heat transfer using a linear regression of the cooling profile.

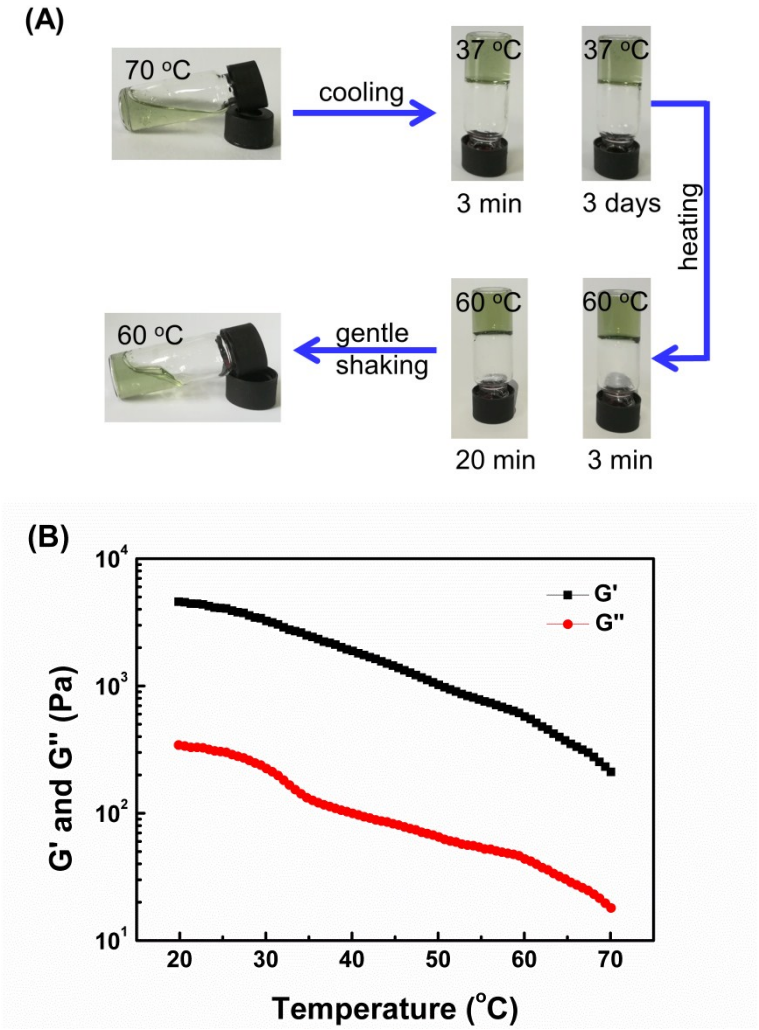

Fig. S3. (A) The sol-to-gel and gel-to-sol transition of NC hydrogel at different temperatures. (B) Temperature-dependence of storage and loss moduli of NC hydrogel at a heating rate of 1 °C/min.

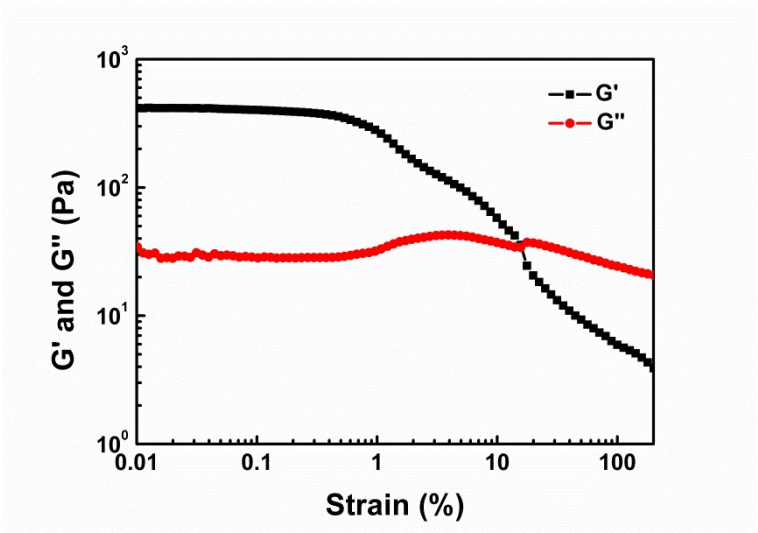

Fig. S4. Storage and loss moduli of NC hydrogel by continuous-strain sweep at 37 ^{o}C.

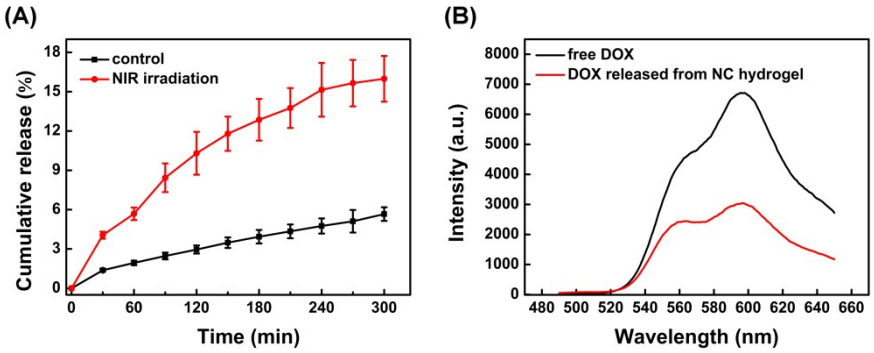

Fig. S5. (A) In vitro releases profiles from DOX-loaded NC hydrogel at pH 6.8 (n = 3). The hydrogel without exposure to laser was used as a control. (B) Fluorescence spectra of DOX in the NC hydrogel after NIR-triggering release.

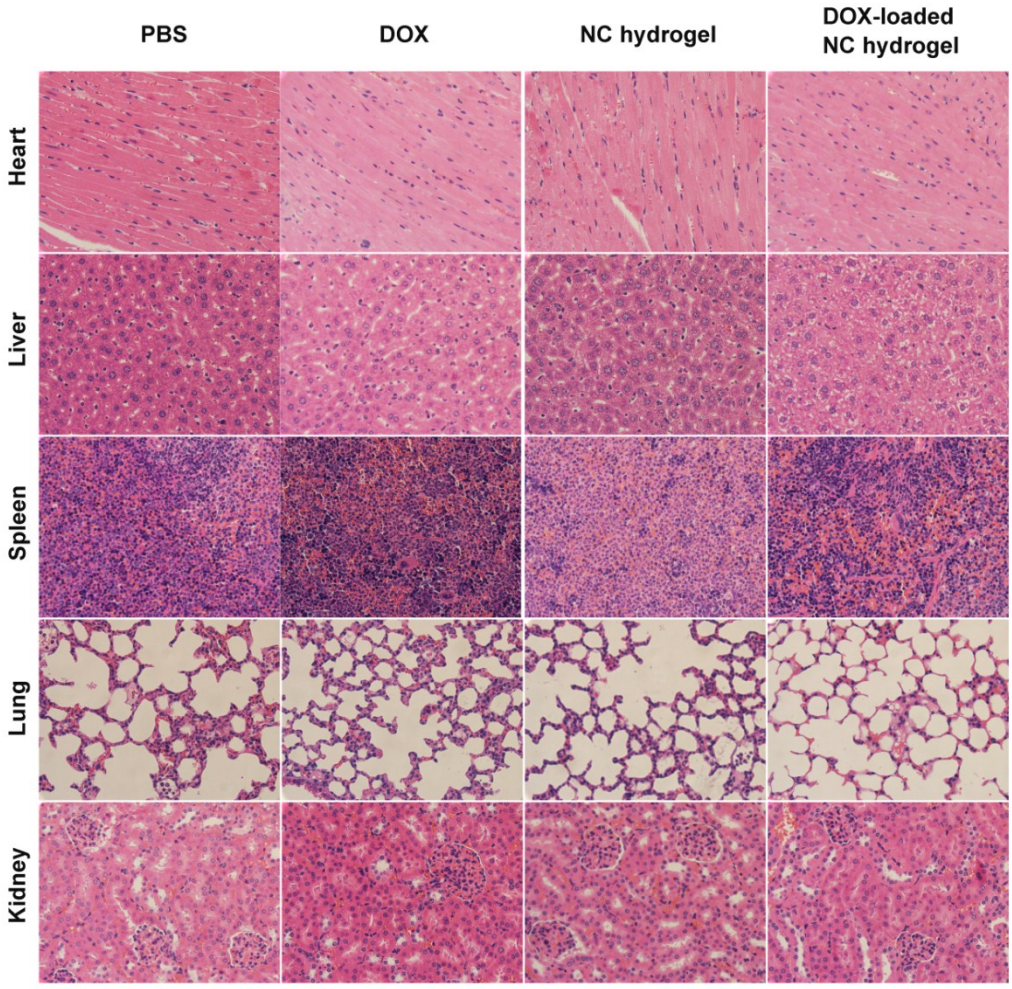

Fig. S6. H&E stained images of major organs collected from mice.

References

1 M. Zhou, R. Zhang, M. Huang, W. Lu, S. Song, M. P. Melancon, M. Tian, D. Liang and C. Li, *J. Am. Chem. Soc.*, 2010, **132**, 15351-15358.

2 C. Sun, L. Wen, J. Zeng, Y. Wang, Q. Sun, L. Deng, C. Zhao and Z. Li, *Biomaterials*, 2016, **91**, 81-89.

3 Z. X. Tu, H. S. Qiao, Y. T. Yan, G. Guday, W. Chen, M. Adeli and R. Haag, *Angew. Chem., Int. Ed.*, 2018, **57**, 11198-11202.