Supplementary Information

Dual Gate-Controlled Intelligent Nanoreactor Enables Collaborative Precise Treatment for Cancer Nanotherapy

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1. Experimental Section

1.1 Materials

HA was purchased from Shangdong Freda Biopharm Co., Ltd. Copper chloride dihydrate ($CuCl_2.2H_2O$), sodium hydroxide (NaOH), sodium sulfide nonahydrate ($Na_2S.9H_2O$), disulfiram (DSF), PEI, and hydrazine were obtained from Sigma-Aldrich (USA). Fe(III) acetylacetonate ($Fe(acac)_3$, 98%), Pt(II) acety-lacetonate ($Pt(acac)_2$, 98%), and Mn(III) acetylacetonate ($Mn(acac)_3$, 98%) were purchased from Beijing HWRK Chem. OA (>85%), OAm (90%), benzyl ether (>98%), 1,2-hexadecanediol (>98%). Alexa Fluor 488 Annexin V/PI Cell Apoptosis Kit and Calcein-AM/Propidium Iodide (PI) Staining Kit were purchased from Invitrogen (USA). N-(3-Dimethylaminopropyl)-N′- ethylcarbodiimide hydrochloride crystalline, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and dimethyl sulfoxide (DMSO) were supplied by Shanghai Aladdin Bio-Chem Technology Co., Ltd. (China). The Calcein-AM/Propidium Iodide (PI) Staining Kit was purchased from Invitrogen (USA). All other chemicals used were analytical reagent grade and were used without further purification.

1.2 Synthesis of HCuS

Specifically, $CuCl_2{\cdot}2H_2O$ was added to water containing HA, followed by introducing 28 mL of NaOH into the previous solution. After stirring for 5 min, hydrazine (30 µl) was dropwise added to the above mixture. Subsequently, Na_2S aqueous solution was added to the orange suspension and heated at 60 °C for 2 h. After the reaction, the HCuS were collected by centrifugation (11000 rpm, 8 min) and washed with deionized (DI) water for (twice) cycles.

1.3 Synthesis of FePtMn Nanoparticles

The FePtMn NCs were synthesized by one-pot thermo-reduction reaction. $Pt(acac)_2$ (0.5 mmol), $Fe(acac)_3$ (1 mmol), $Mn(acac)_3$ (1 mmol) and 1,2-hexadecanediol

were dissolved in benzyl ether (30 mL). After degassing and backfilling with argon, the solution was heated to 100 °C. Then, OA (0.17 mL) and OAm (0.16 mL) were added and heated to reflux (295 °C) for 60 min. The product was collected by centrifugation (12000 rpm for 6 min) with hexane and ethanol.

1.4 Characterizations

Transimission election microscopy (TEM) images, high-resplution TEM (HRTEM) images were determined on a JEOL-2100 transmission electron microscope. High-angle annular dark-field (HAADF)-scanning transmission electron microscopy (STEM) coupled with EDS elemental mapping was conducted on a JEOL ARM-200F field emission transmission electron microscope. Dynamic light scattering (DLS) and Zeta potential were detected by Nano-ZS (Malvern). The content of iron, platinum, manganese and copper was determined on a Thermo Fisher inductively coupled plasma (ICP) optical emission spectrometry (iCAP Qc). The UV-vis spectra were carried out on a NANO DROP 2000 spectrophotometer, respectively. X-ray photoelectron spectroscopy (XPS) measurement was performed to analyse the elemental composition and binding energy of samples on a Thermo Scientific ESCALAB 250 Xi. The crystal structure of obtained catalysts were characterized by X-ray diffractometer (XRD).

2. Experimental Methods

2.1 O_2 Generation Study

The O_2 generation was investigated at different mixed condition of H_2O，H_2O_2 (10 mM) or HDHF within 12 min by a dissolved oxygen meter.

2.2 In Vitro Photothermal Performance of HDHF

Different concentrations of HDHF were exposed to laser irradiation at 808 nm (1.25 W cm^{-2}). In addition, we irradiated HDHF solutions with 808 nm laser irradiators at different power intensities. The temperature change of HDHF under 808 nm laser irradiation was observed by infrared thermal imaging recorder.

2.3 Cell Culture

4T1 cells were incubated in DMEM medium involving 10% FBS, 20 mM HEPES, 2 mM L-glutamine, 100 U/mL streptomycin and 100 U/mL penicillin at 37°C under 0.1% O_2 and 5% CO_2.

2.4 Determination of ROS generation in vitro

The fluorescent probe of 2′, 7′-dichlorodihydrofluorescein diacetate (DCFH-DA) reagent were used to detect ROS. After incubation with PBS, HCuS (30 μg mL^{-1}), HCuS@FePtMn (30 μg mL^{-1}), HDHF (30 μg mL^{-1}), HCuS (30 μg mL^{-1}) + 808nm Laser and HDHF (30 μg mL^{-1}) + 808nm Laser for 6 h, the 4T1 cells were washed twice with PBS. Then, 1 mL of DMEM containing DCFH-DA and Hochest 33342 was added, and the cells were cultured for another 30min. After being washed thrice with PBS, 4T1 cells stained with DCFH-DA (green) and Hoechst 33342 (blue) were fluoresced under an inverted microscope.

2.5 Evaluation of the cytotoxicity of HDHF

4T1 cells were placed into 96-well plates at a density of 1 × 10^4 cells/well and incubated overnight. Then, different concentrations of HDHF were added to the corresponding wells. After 12 h incubation, cells were treated with 10ul of MTT solution (5 mg/ml). Incubate for an additional 4 h, remove the medium from each well, and add 100 ul of dimethyl sulfoxide (DMSO) to dissolve the formazan crystals. Finally, the absorbance at 570 nm was recorded using a microplate reader. Untreated cells were used as a control group. Cell viability was calculated according to the equation shown below:

$$Cell\ viability\ (\%) = \frac{A_{sample} - A_{blank}}{A_{control} - A_{blank}} \times 100\%$$

2.6 Live/dead staining assay

The 4T1 tumor cells were cultured in 6-well plates and exposed to various treatments, including PBS, DSF, FePtMn, HCuS, HCuS@FePtMn, HDHF, HCuS +

808nm Laser and HDHF + 808nm Laser. Laser exposure (1.25 W cm^{-2}) was implemented for 5 min. After being washed thrice with PBS, 4T1 cells were incubated with calcein-AM/PI. Finally, 4T1 cells were washed thrice with PBS and fluoresced under an inverted microscope.

2.7 Hemolysis Assay

Blood samples (0.1 mL) were donated from a single mouse. Red blood cells (RBCs) were collected by centrifugation at 3000 rpm for 5 min, washed 3 times with PBS and resuspended with PBS (10 mL) to prepare a RBC suspension. Then, different concentrations of HDHF (10, 20, 40, 60, 80 and 100 μg/mL; PBS was the solvent) were mixed with RBC suspensions in medium volumes in centrifuge tubes, respectively. After incubation at 37°C for 12 h, the supernatant was centrifuged to obtain the supernatant. The RBCs in PBS and water were used as negative and positive controls, respectively.

2.8 In vivo fluorescence imaging

Cy7-labeled HDHF was intravenously injected into balb/c mice with tumors.[1] The mice were then placed in an imaging device and fluorescence images were obtained at various time intervals after injection. The mice were euthanized 12 hours after injection, and fluorescence images and fluorescence intensity of major organs were obtained.

2.9 In Vivo Synergistic Anticancer Study

All animal procedures were performed in accordance with the Guidelines for Care and Use of Laboratory Animals of Linyi University and experiments were approved by the Animal Ethics Committee of Linyi University.

To assess the treatment effect, 4T1 tumor-bearing balb/c mice with tumor volumes of 80-100 mm^3 were selectively divided into 9 groups (n = 5) and treated with PBS, PBS + 808 nm laser (1.25 W cm^{-2}), DSF, HCuS, FePtMn, HCuS@FePtMn, HCuS+ 808 nm laser (1.25 W cm^{-2}), HDHF and HDHF + 808 nm laser (1.25 W cm^{-2}) treatments.

HCuS, HCuS@FePtMn and HDHF in PBS (50 μL) at a Cu dose of 20 mg kg^{-1} were intravenously administrated only once before performing laser irradiation. PBS solution containing nanomaterial was injected intravenously into the mice through the tail vein every 2 days. The tumor volume and weight of the mice were recorded before the next injection. After treatment, mice were euthanized, and major organs and tumors were obtained for terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL), hematoxylin and eosin (H&E) staining, and HIF-1α staining.

2.10 Calculation of the photothermal conversion efficiency

The photothermal conversion efficiency of the HDHF was determined according to the previous method.[2, 3] The η value was calculated as follows:

$$\eta = \frac{\mathrm{h}S(Tmax - Tsurr) - Q0}{I(1 - 10 - A\lambda)}$$

where h is the heat transfer coefficient, S is the surface area of the container, T_{max} and T_{surr} are the equilibrium temperature and ambient temperature, respectively. Q_0 is the heat associated with the light absorbance of the solvent, A_λ is the absorbance of the HDHF at 808 nm, and I is the laser power density.

According the formula:

$$\theta = \frac{T - Tsurr}{Tmax - Tsurr}$$

$$t = -\tau s Ln(\theta)$$

$$\mathrm{h}S = \frac{mdCd}{\tau s}$$

Where τ_s is the time constant of the sample system, m_d and C_d are the mass of water and the heat capacity, respectively.

According to Fig. 2i, τ_s was determined and calculated to be 244.3 s.

Thus, substituting according values of each parameters into

$$\eta = \frac{\mathrm{h}S(Tmax - Tsurr) - Q0}{I(1 - 10 - A\lambda)}$$

the photothermal conversion efficiency (η) of the HDHF could be calculated to be 46.8%.

3. Supplementary Figures

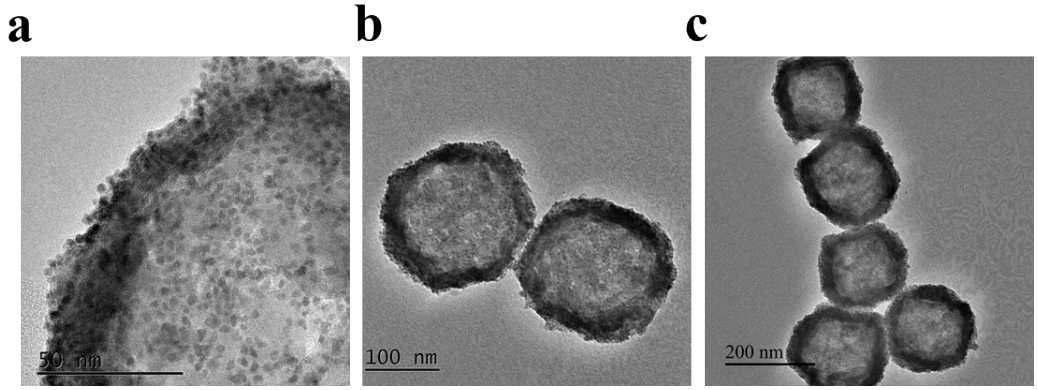

Fig. S1 TEM images of HDHF at different magnifications.

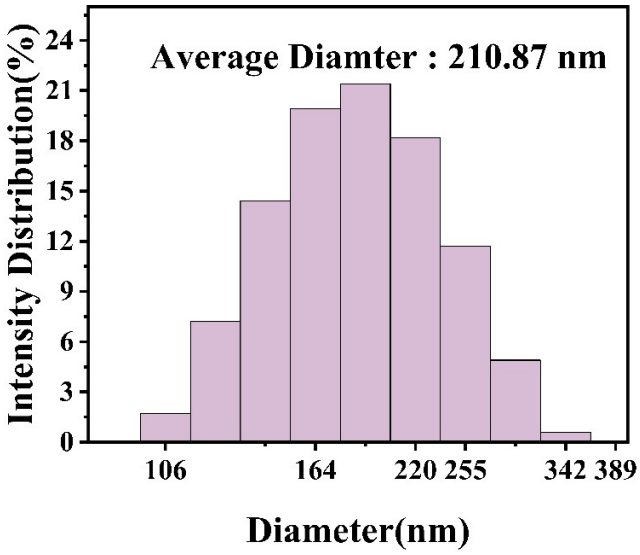

Fig. S2 Size distribution of PEI-HCuS@FePtMn by DLS measurement.

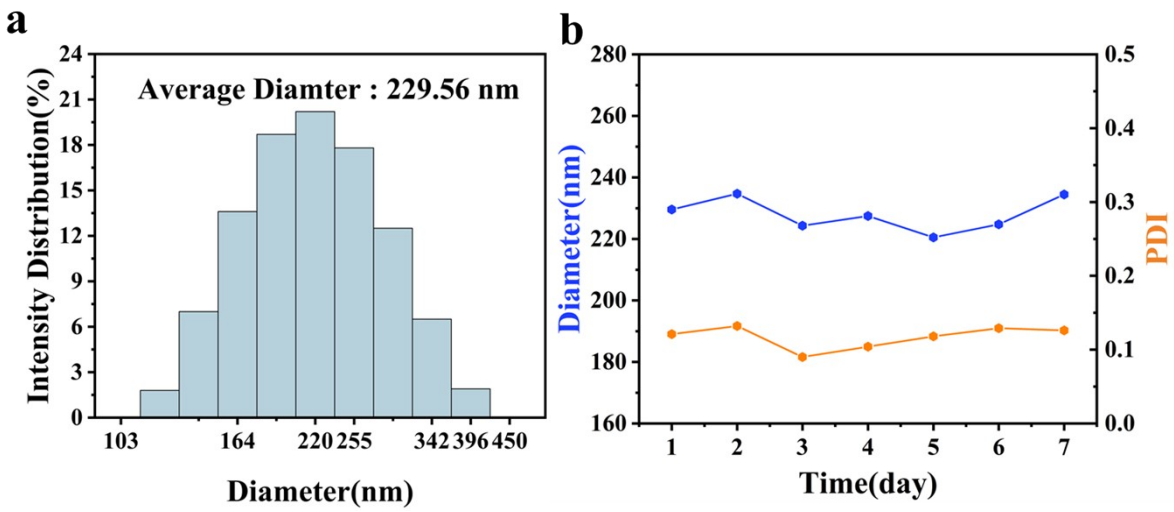

Fig. S3 (a) Size distribution of HDHF by DLS measurement. (b) The changes of hydrodynamic size and PDI of HDHF size distribution and PDI changes of HDHF within 7 days.

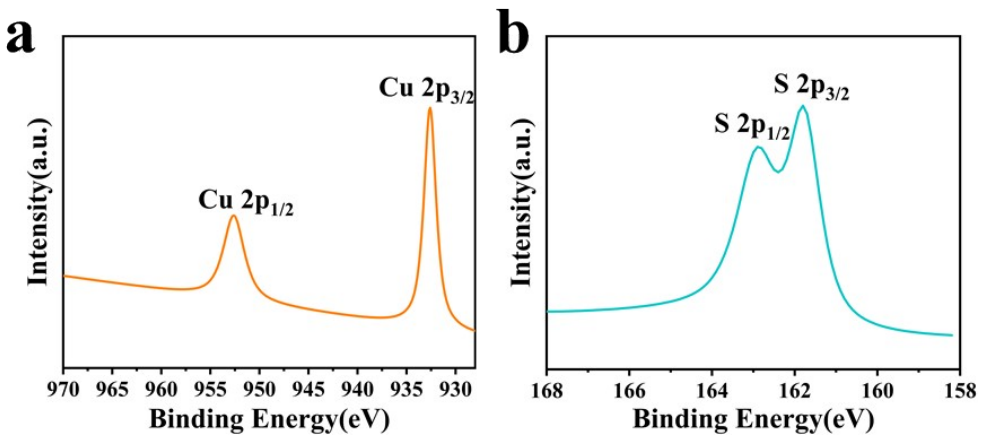

Fig. S4 (a,b) high-resolution Cu 2p and S 2p XPS spectra of HCuS.

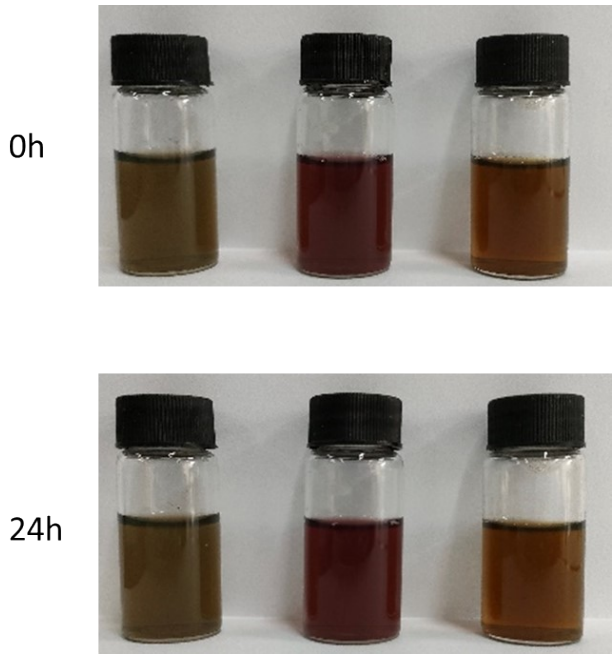

Fig. S5 The stability image dispersed in PBS, FBS and DMEM of HDHF.

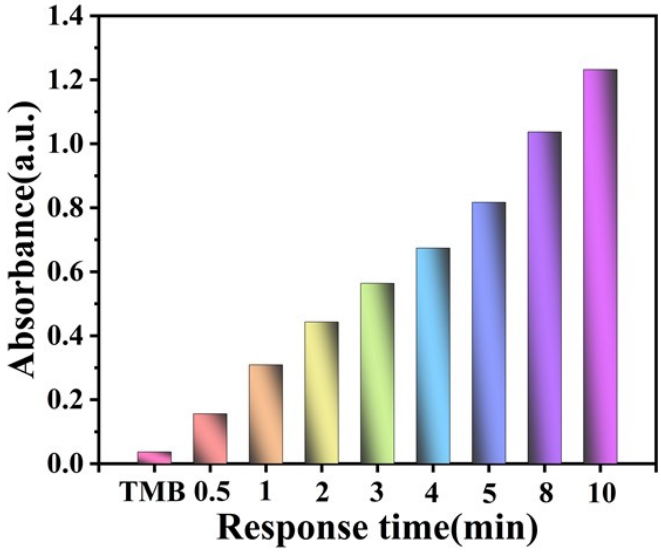

Fig. S6 The relationship between the ability of HDHF to generate hydroxyl radicals at pH=5.8 and the time.

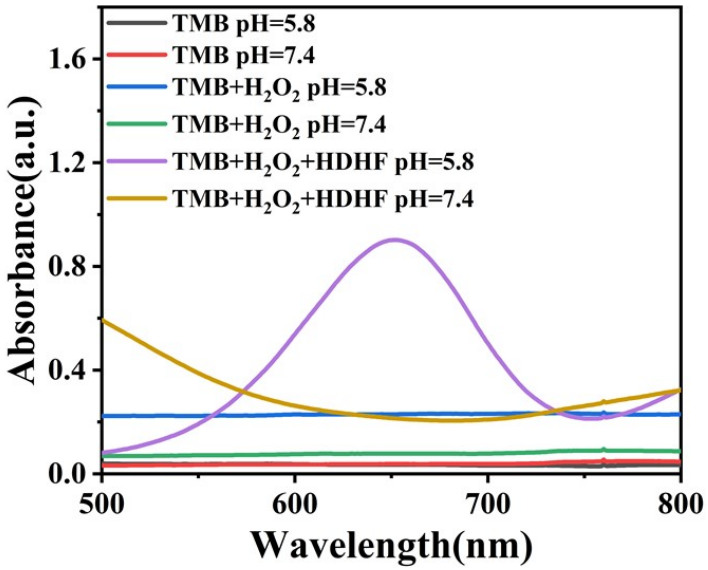

Fig. S7 The ability of HDHF to generate ROS in solutions with different pH values.

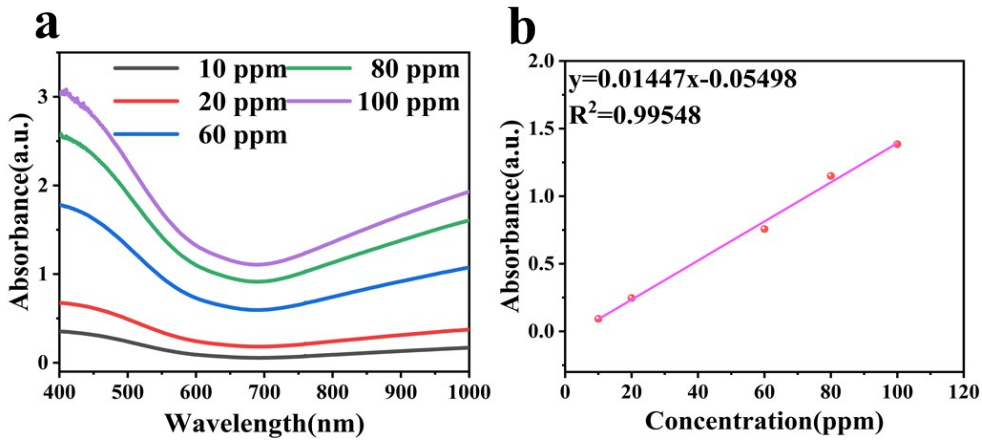

Fig. S8 (a) UV−vis−NIR absorption spectra of varying doses of HCuS (10, 20, 60, 80, and 100 ppm). (b) The fitting curve of extinction coefficient of HCuS at 808 nm.

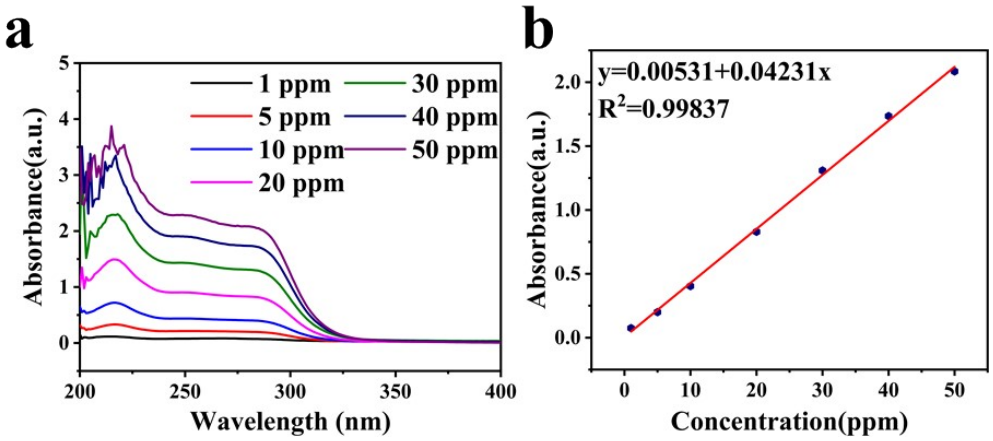

Fig. S9 (a) UV absorption peaks for different concentrations of DSF (1,5,10,20,30,40 and 50 ppm). (b) The working curves were prepared based on the absorbance at 280 nm for different concentrations of DSF.

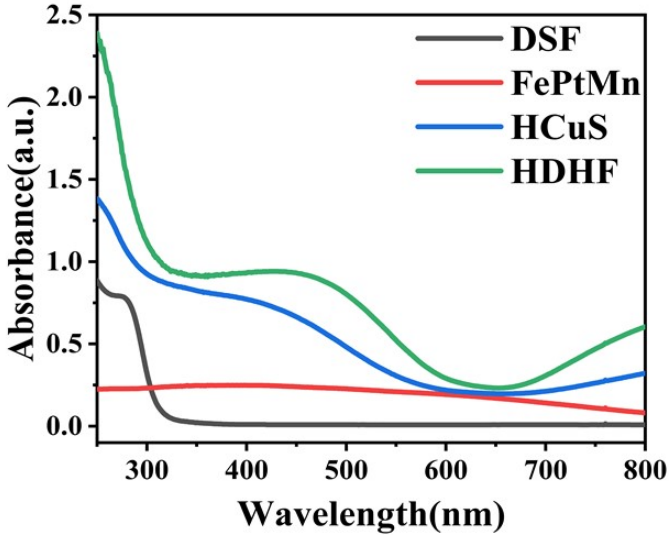

Fig. S10 UV−vis−NIR absorption spectra of DSF, FePtMn, HCuS and HDHF.

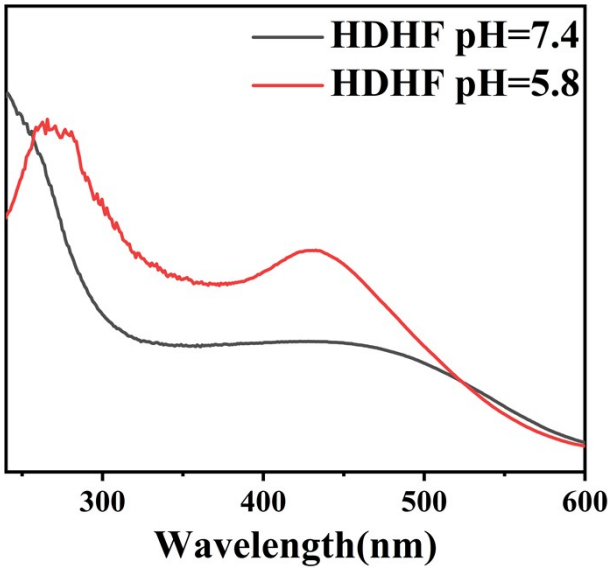

Fig. S11 Formation of $Cu(DTC)_2$ from HDHF after immersion in SBF at different pH values.

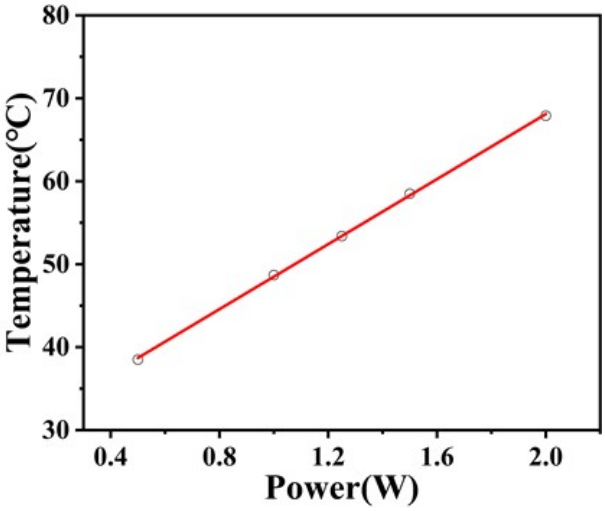

Fig. S12 Variation of the reached plateau temperature with the laser power.

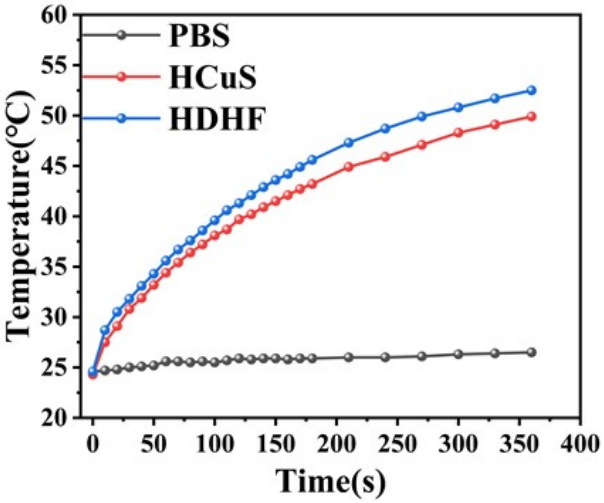

Fig. S13 Temperature profiles of PBS, HCuS and HDHF exposed to 808 nm laser with time.

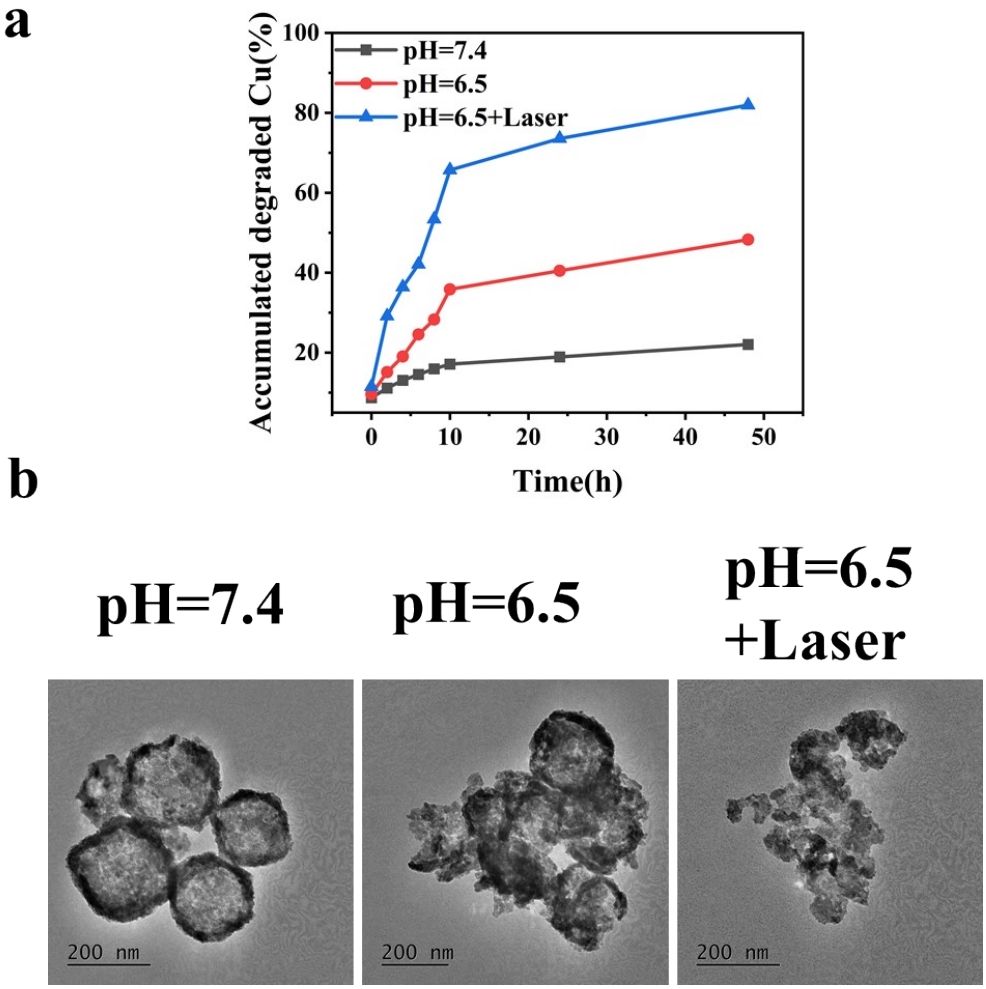

Fig. S14 (a) Accumulative releasing profiles of biodegraded Cu^{2+} from HDHF in SBF solution (pH = 6.5 or 7.4) in the absence and presence of 808 nm laser irradiation. (b) TEM images of HDHF after 12 h incubation in SBF solution (pH=6.5 or 7.4) without and with 808nm laser irradiation

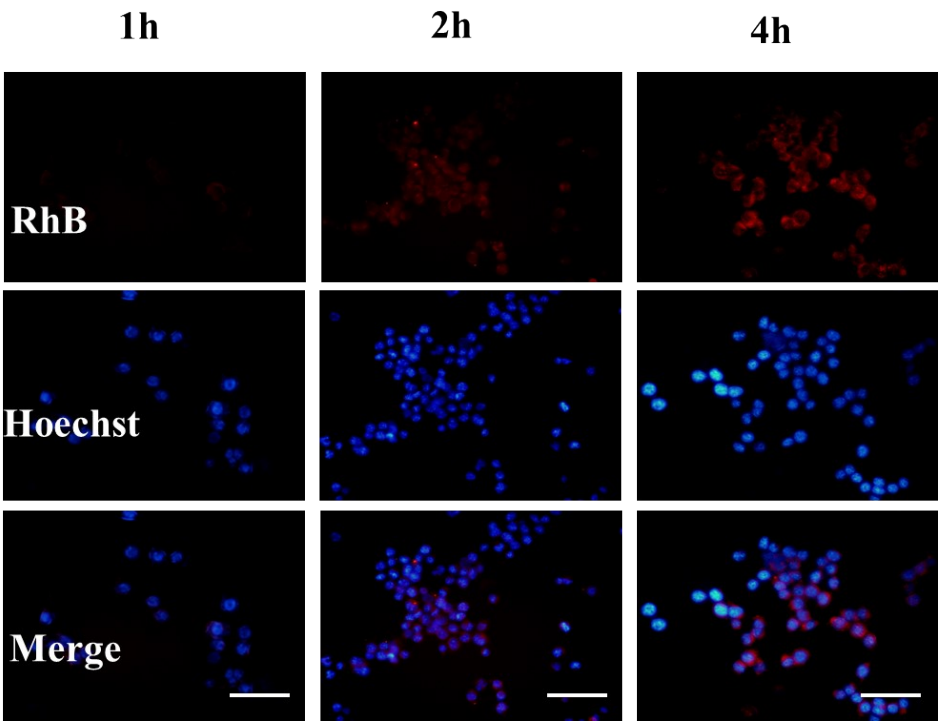

Fig. S15 Fluorescence microscopy images showing the uptake of RhB@HDHF by cells after co-culture with 4T1 cells for 1, 2 and 4 hours (scale bar: 50 μm).

Fig. S16 Fluorescence images of L02 cells incubated with RhB@HDHF for 4 h (scale bar: 100 μm).

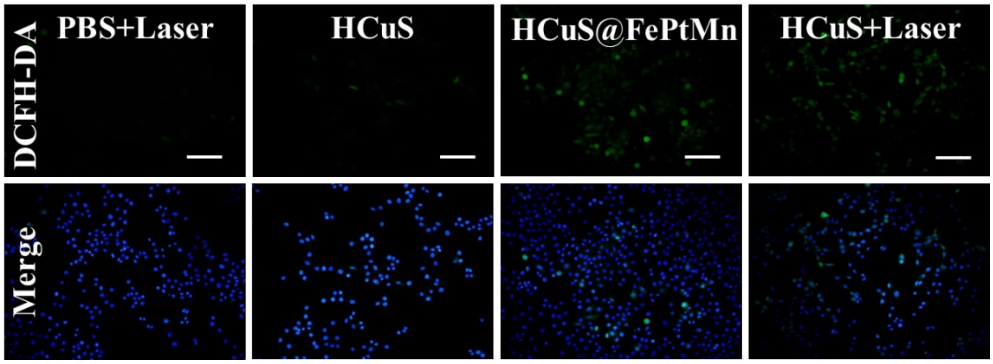

Fig. S17 ROS fluorescence imaging of 4T1 cells treated with DCFH-DA and Hoechst 33342 in diffffferent groups (scale bar: 100 μm).

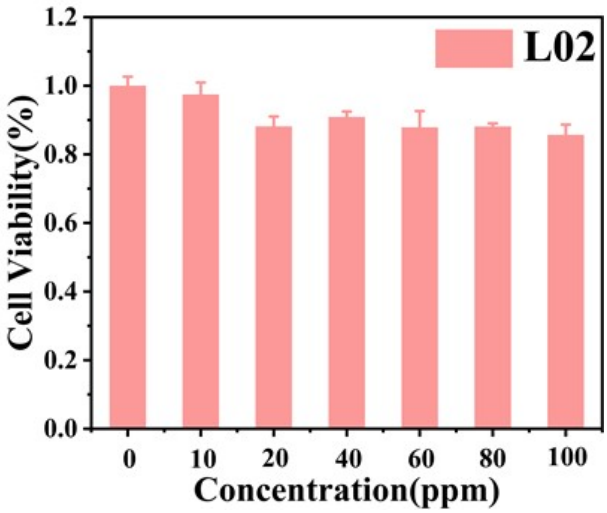

Fig. S18 The effects of different concentrations of HDHF incubation for 12 h on the viability of L02 cells.

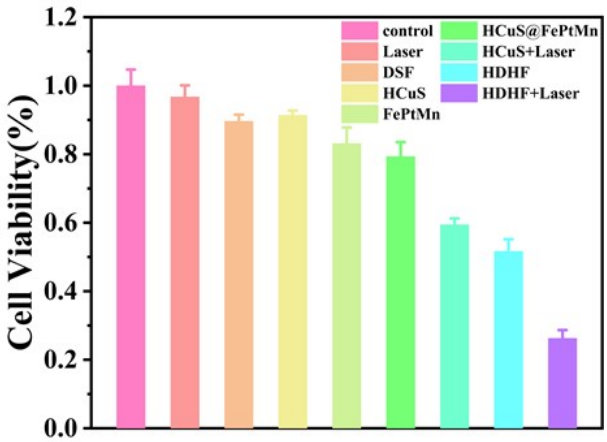

Fig. S19 4T1 cell viability after different treatments, including PBS, PBS+Laser, DSF, HCuS, FePtMn, HCuS@FePtMn, HCuS + Laser, HDHF and HDHF+ Laser groups (1.25 W cm^{-2}).

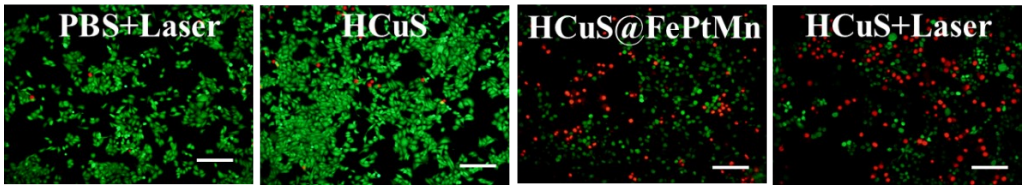

Fig. S20 Fluorescence microscopy images of 4T1 cells labeled with calcein-AM and PI (scale bar: 100 μm).

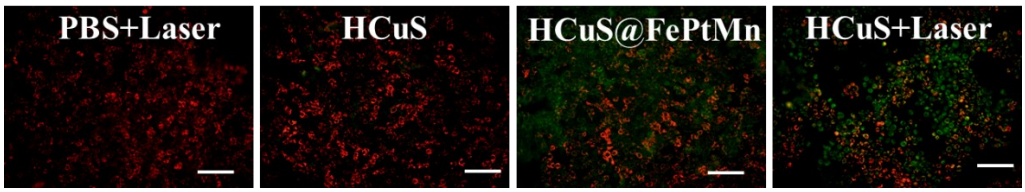

Fig. S21 The detection of intracellular mitochondrial membrane potential with JC-1 dye (scale bars: 100 μm).

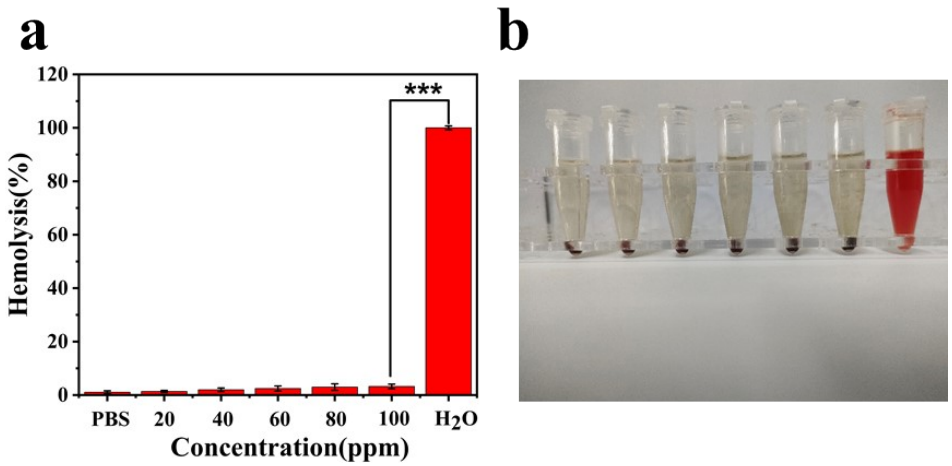

Fig. S22 (a) Photographs of RBC suspensions treated with different concentrations of HDHF. RBC suspensions treated with PBS and H_2O were set as negative and positive controls, respectively. (b) Corresponding hemolysis rates of RBC samples in (a).

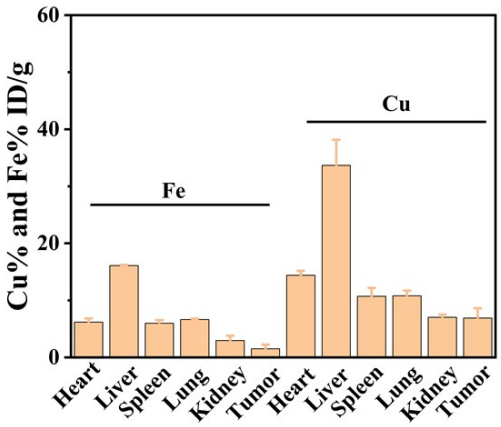

Fig. S23 The distribution of Cu and Fe in the major organs (heart, liver, spleens,lungs, and kidney) and tumors of 4T1 tumor-bearing female balb/c mice after HDHF treatment for 36 h.

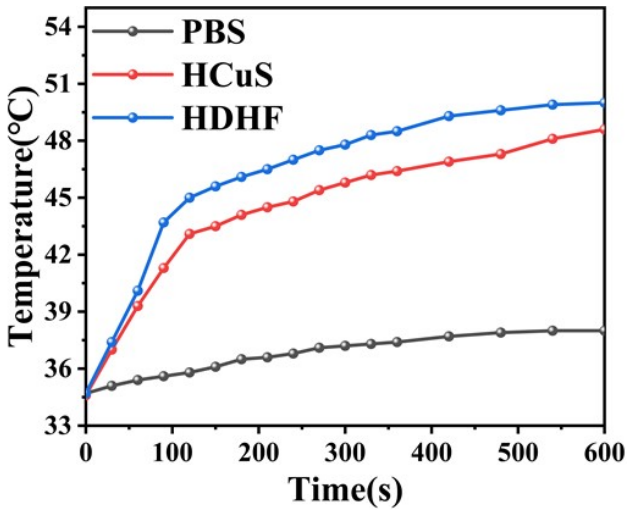

Fig. S24 Temperature-elevation curves of tumors after treatment with HDHF under 808 nm exposure (1.25 W cm^{-2}) at varying treatment durations.

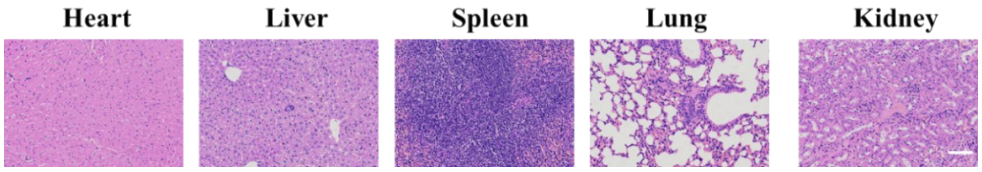

Fig. S25 H&E stained images of dissected major organs derived from the mice after treatment with HDHF+Laser for 14 days (scale bars: 100 μm).

Supplementary References

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