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Supporting Information

Synthesis of porphyrin-incorporated covalent organic frameworks for sonodynamic therapy

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Experimental Section

Chemicals and Materials. 1,3,5-tris(4-aminophenyl)benzene ($C_{24}H_{21}N_3$, AR, 98%, Alpha), 2,5-dimethoxyterephthaldehyde ($C_{10}H_{10}O_4$, AR, Jilin Chinese Academy of Sciences-Yanshen Technology Co. Ltd), 4,4',4",4"'-(21H,23H-porphine-5,10,15,20-tetrayl)tetrakis-Benzaldehyde ($C_{48}H_{30}N_4O_4$, AR, Jilin Chinese Academy of Sciences-Yanshen Technology Co. Ltd), Hyaluronic acid ($C_{28}H_{44}N_2O_{23}$, Shanghai Macklin Biochemical Co. Ltd), Acetonitrile (CH_3CN, 99.8%, Vetec), Acetic acid (CH_3COOH, AR, Beijng Chemical Works), Ethanol (AR, Beijing Chemical Works), Trichloromethane (AR, Beijing Chemical Works).

Characterization. Powder X-ray diffraction (PXRD) studies were performed on a Rigaku MiniFlex 600 diffractometer with graphite monochromatized CuKα radiation (λ = 0.15405 nm). The sample was scanned at a scanning rate of 10°/min in the 2θ range from 2 to 20 ° at room temperature. Field emission scanning electron microscope (FE-SEM, S-4800, Hitachi) equipped with an energy dispersive X-ray (EDX) spectrometer was used to characterize the morphology of the sample. Thermogravimetric analysis data was recorded on a TGA 500 thermogravimetric analyzer by heating with a rate of 10 ℃/min under the nitrogen atmosphere (60 mL/min). Fourier transform infrared spectroscopy (FT-IR) was measured on a Vertex PerkinElmer 580 BIR spectrophotometer (Bruker) using the KBr tabletting technique. The sample was securely packaged to obtain a transparent film. The UV-Vis adsorption spectral values were obtained on a U-3310 spectrophotometer (Hitachi). Dynamic light scattering (DLS) experiment was measured by Malvern Zeta Sizer-Nano ZS90 instrument at 25 ℃. MTT experiments were carried out using a microplate reader (Thermo Multiskan MK3).

Synthesis of TAPB-DMTP-COF. 0.8 mg (0.0048 mmol) of 2,5-dimethoxyterephthaldehyde (DMTP), 1 mg (0.0028 mmol) of 1,3,5-tris(4-aminophenyl)benzene (TAPB) and 2 mL of acetonitrile were mixed with ultrasound, followed by the addition of 0.05 mL of acetic acid and the mixture was stirred at room temperature for 12 h. The precipitate was collected by centrifugation and washed with ethanol for three times.

Synthesis of CPF. 2 mg of 1,3,5-tris(4-aminophenyl)benzene (TAPB) and 1 mg of 2,5-dimethoxyterephthaldehyde (DMTP) were dissolved in 2 mL acetonitrile, 1 mg 4,4',4",4"'-(21H,23H-porphine-5,10,15,20-tetrayl)tetrakis-Benzaldehyde was dissolved in 2 mL trichloromethane and the two solutions were mixed, followed by the addition of 0.1 mL acetic acid and then held for 3 h. The precipitate was collected by centrifugation and washed with ethanol for three times.

Synthesis of CPA. 10 mg of CPF was dispersed in 10 mL of deionized water and then added with 5 mg of HA. After stirring for 12 h, the product was collected by centrifugation and washed with deionized water for three times.

Sonodynamic effect of COF and CPF. To study the singlet oxygen generation capacity of COF and CPF under US irradiation, 1,3-Diphenylisobenzofuran (DPBF) was used as an indicator. 2 mg of COF or CPF aqueous solution was added to the cuvette, and then 10 μL of DPBF in dimethyl

sulfoxide with a concentration of 10 mg/mL was added. The mixed solution was irradiated by US (1 MHz, 50% duty cycle, 1.5 W cm^{-2}). The absorption wavelength of DPBF near 410 nm after being irradiated for different times was monitored by ultraviolet-visible spectroscopy. The capacity of COF and CPF to generate singlet oxygen was estimated by the degree of reduction in absorption intensity.

Intracellular ROS Detection. 4T1 cells were incubated with CPA (50 μg mL^{-1}) for 24 h, and treated with US irradiation (1 MHz, 50% duty cycle, 1.5 W cm^{-2}, 1 min). Then the culture medium was replaced and RPMI containing DCFH-DA (10×10^{-6} M) was added and further incubated in the dark for 20 min. Then it was washed by PBS for three times and observed under a fluorescence microscope.

Cellular Internalization of CPA. In order to study the cellular uptake of CPA, rhodamine B (RhB)-loaded CPA was prepared. 10 mg of 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC), 3 mg of N-Hydroxysuccinimide (NHS) and 5 mg of rhodamine were dissolved in 5 mL of deionized water, stirred at room temperature for 1 h, and then 5 mL of CPA solution in deionized water (about 5 mg) was added and stirred overnight. The product was washed with deionized water until the supernatant was nearly colorless. 4T1 cells were incubated with rhodamine B (RhB)-loaded CPA (50 μg/mL) for 1, 2, 4 h, respectively. It was then washed with PBS for three times. The nuclei were labeled with 4,6-diamino-2-phenylindole (DAPI) for 10 min and then observed under a fluorescence microscope.

In vitro biocompatibility of CPA. In order to study the in vitro biocompatibility of CPA, L929 cells were seeded into 96-well plates with a density of 6000 cells per well and cultured in Dulbecco's modified eagle medium (DMEM) for 24 h. The original medium was then sucked out and the new medium containing different concentrations of CPA was added again. After the cells were incubated for another 24 h, the medium inside was again discarded and re-added with fresh DMEM containing 10 μL of MTT. After placing the plates in the dark for 4 h, 150 μL of DMSO was added to each well and the absorption value of the medium was measured by a microplate reader at the wavelength of 490 nm.

In vitro cytotoxicity of COF and CPA. For the in vitro cytotoxicity test, four groups of 4T1 cells were seeded into 96-well plates with a density of 6000 cells per well and cultured in RPMI for 24 h. The original medium was then sucked out and the new medium containing different concentrations of COF and CPA was added again, respectively. After the cells were incubated for 4 h, the medium inside was again discarded and readded with fresh RPMI. Two groups of 4T1 cells were not treated and the other two groups were treated with US irradiation (1 MHz, 50% duty cycle, 1.5 W cm^{-2}, 1 min). Then the cells were incubated for another 24 h before the MTT assay was used to detect the cell viability.

Cell Apoptosis of COF and CPA Nanoparticles. To study the cell apoptosis process of, the Annexin V-FITC/PI Apoptosis Detection Kit was used. 4T1 cells were treated with PBS, US (1 MHz, 50% duty cycle, 1.5 W cm^{-2}, 1 min), COF (50 μg mL^{-1}), COF + US (50 μg mL^{-1}, 1 MHz, 50% duty cycle, 1.5 W cm^{-2}, 1 min), CPA (50 μg mL^{-1}), CPA + US (50 μg mL^{-1}, 1 MHz, 50% duty

cycle, 1.5 W cm^{-2}, 1 min), respectively, and then the cells were incubated in 6-well plates overnight. After that, the cells were harvested and washed with PBS, and then resuspended with binding buffer (400 μL). At last, 5 μL of Annexin V-FITC and 5 μL of PI were utilized to stain the samples for 15 min and 5 min in the dark, respectively. The cell apoptosis process was monitored via a flow cytometer.

In vivo antitumor therapy. Female Balb/C mice (about 18 g) were purchased from the Center of Experimental Animals, Jilin University (Changchun, China), and the animal experiments agreed with the criterions of The National Regulation of China for Care and Use of Laboratory Animals. The development of tumor model was achieved by subcutaneous injection of 4T1 cells into the right side of Balb/C mice. When the tumor volume reached up to 80-120 mm^3, thirty cancer-bearing mice were randomly divided into six groups, and treated with PBS, US, COF (500 μg mL^{-1}, 100 μL), COF (500 μg mL^{-1}, 100 μL) + US, CPA (500 μg mL^{-1}, 100 μL), CPA (500 μg mL^{-1}, 100 μL) + US, respectively. All groups of mice received twice treatment on day 1 and day 7 by US irradiation (1 MHz, 50% duty cycle, 1.5 W cm^{-2}, 10 min), respectively. The relative tumor volume was V/V_0 and the tumor volume was calculated by $V=4/3 \times length \times width^2/8$, where V_0 was the tumor volume before treatment.

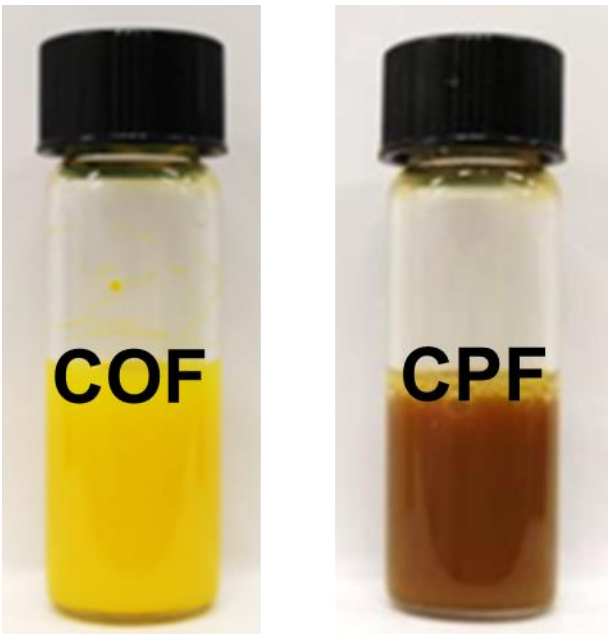

Fig. S1 The photographs of COF and CPF dispersed in deionized water.

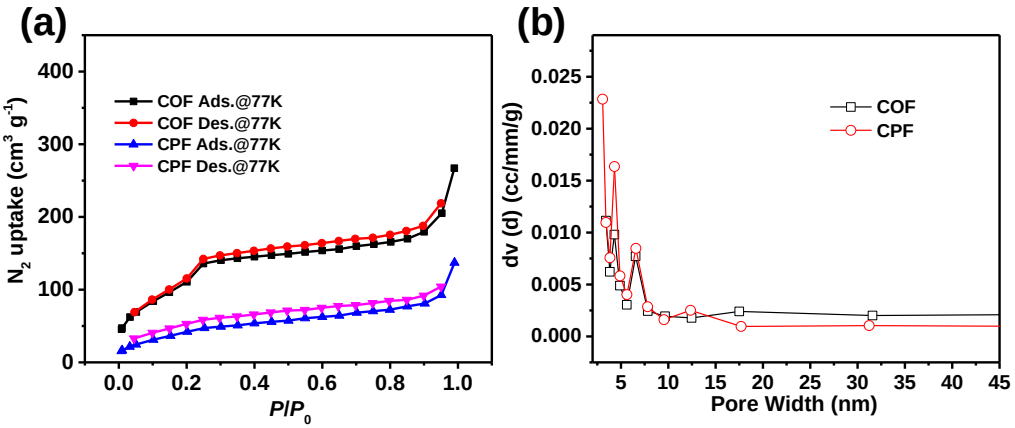

Fig. S2 (a) The N_2 sorption isotherms and (b) pore size distribution curve for COF and CPF.

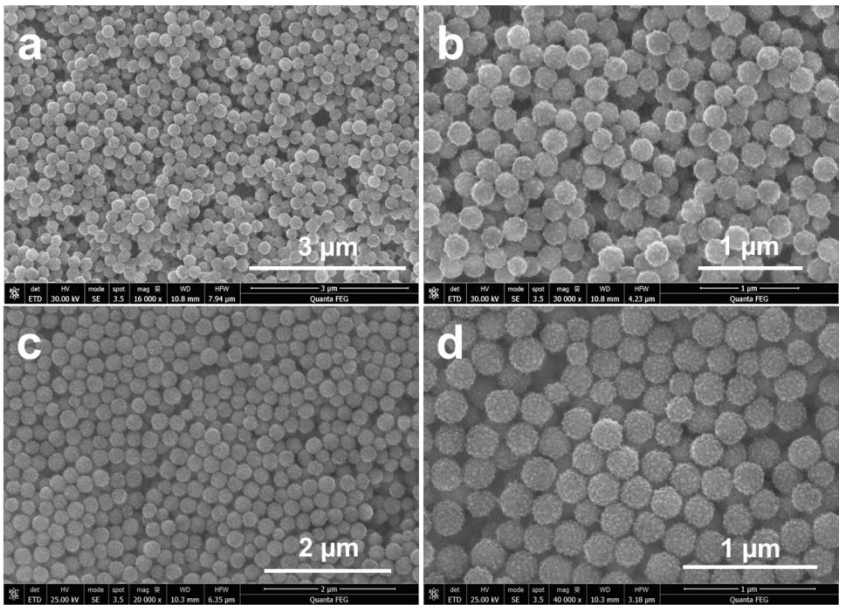

Fig. S3 Additional SEM images of (a and b) COF and (c and d) CPF.

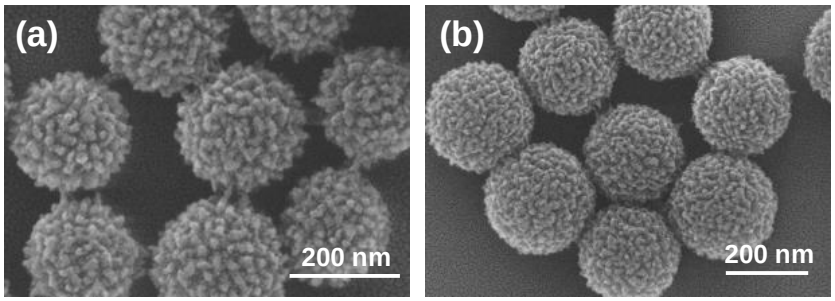

Fig. S4 SEM images of (a) CPF and (b) CPA.

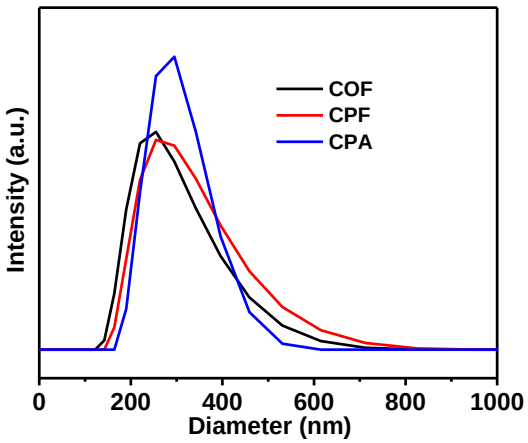

Fig. S5 The DLS results of COF, CPF and CPA, respectively.

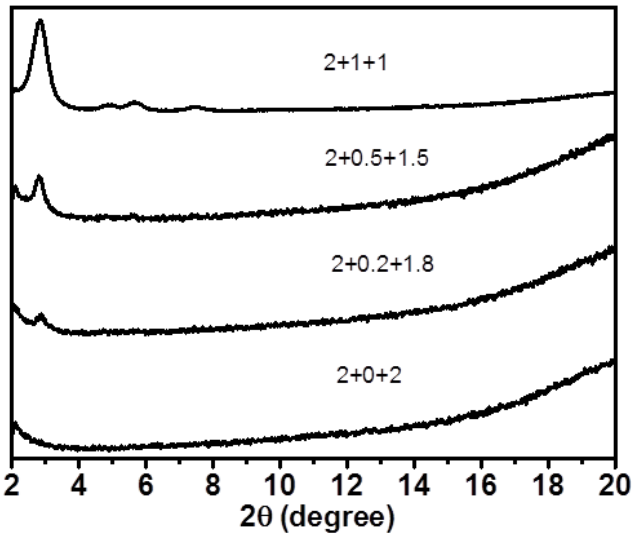

Fig. S6 The PXRD patterns of the four products with different ratios of TAPB:DMTP:TFPP.

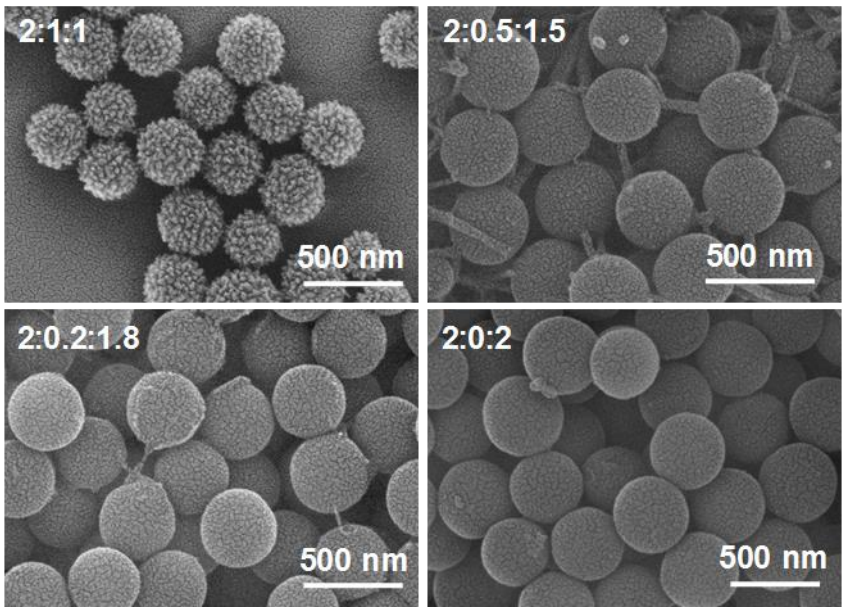

Fig. S7 SEM images of the four products with different ratios of TAPB:DMTP:TFPP.

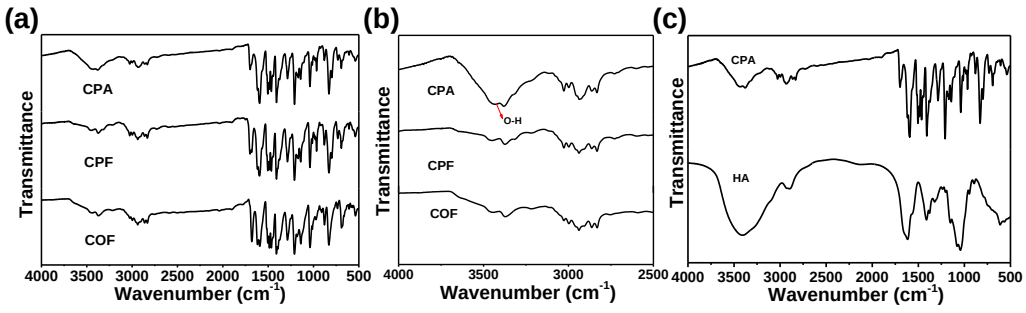

Fig. S8 (a) The FT-IR spectra of COF, CPF and CPA. (b) The enlarged spectra from 4000 cm^{-1} to 2500 cm^{-1}. (c) The FT-IR spectra of HA and CPA.

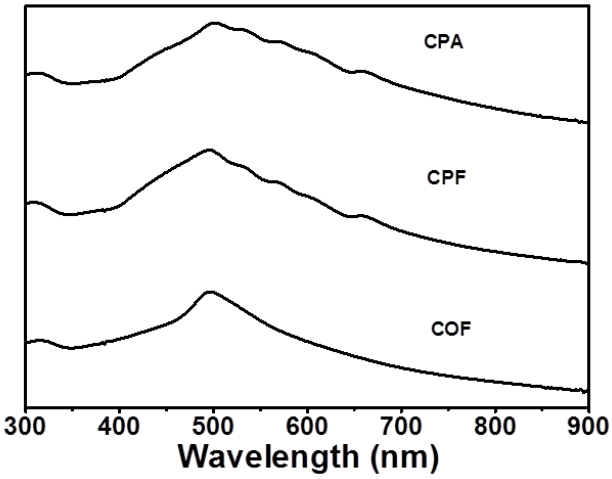

Fig. S9 UV-vis absorption spectra of COF, CPF and CPA, respectively.

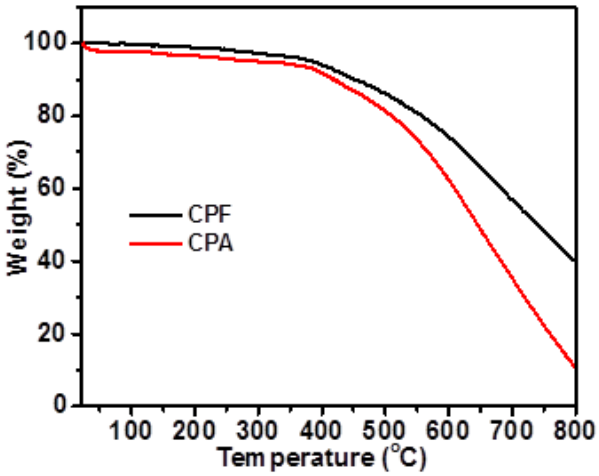

Fig. S10 The TGA curve of CPF and CPA, respectively.

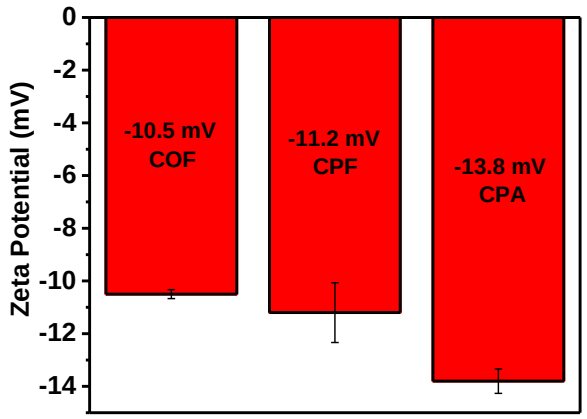

Fig. S11 Zeta potentials of COF, CPF and CPA, respectively.

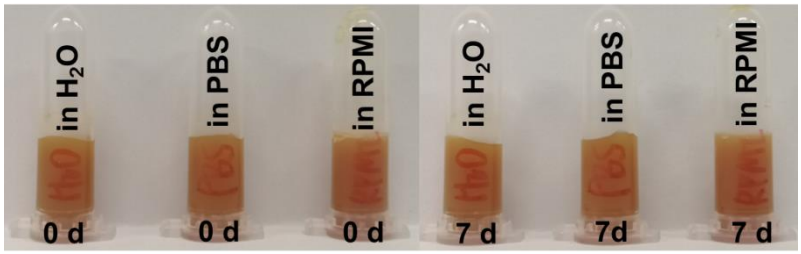

Fig. S12 The photographs of CPF dispersed in H_2O, PBS and RMPI for 0 d and 7 d, respectively.

Fig. S13 The photographs of CPA dispersed in H_2O, PBS and RMPI for 0 d and 7 d, respectively.

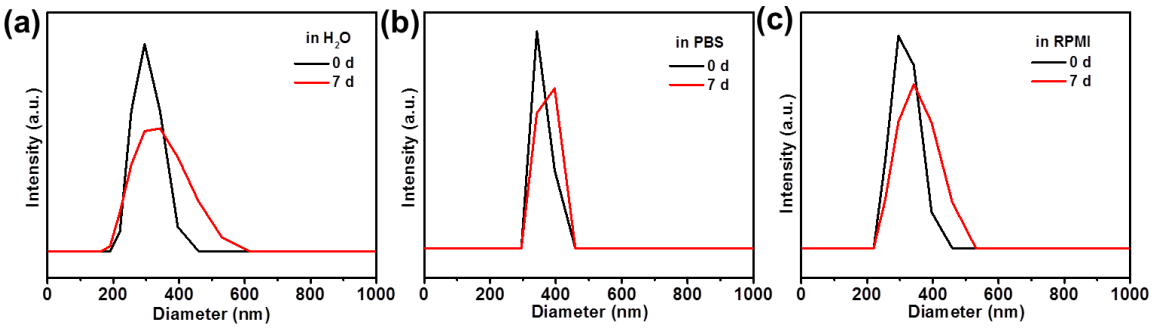

Fig. S14 The DLS results of CPA dispersed in H_2O, PBS and RPMI, respectively.

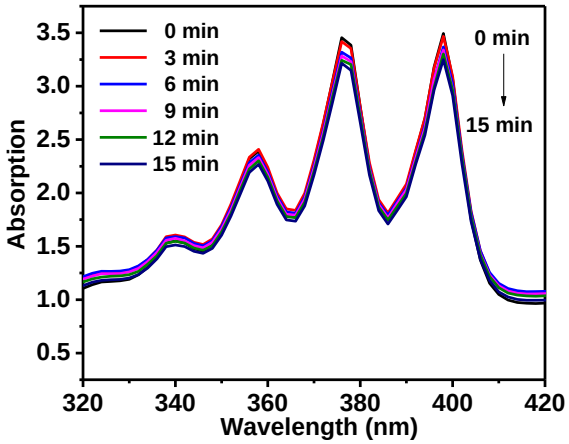

Fig. S15 UV-vis absorption spectra of pure ABDA treated with US irradiation for different times (1 MHz, 50% duty cycle, 1.5 W cm^{-2}).

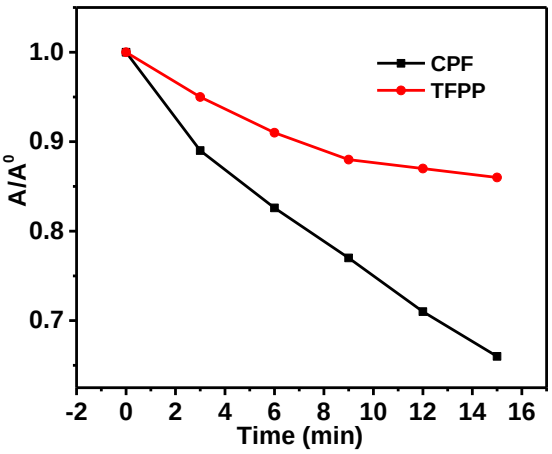

Fig. S16 UV absorption change curve of ABDA in the presence of CPF and TFPP under the same power density of ultrasound irradiation.

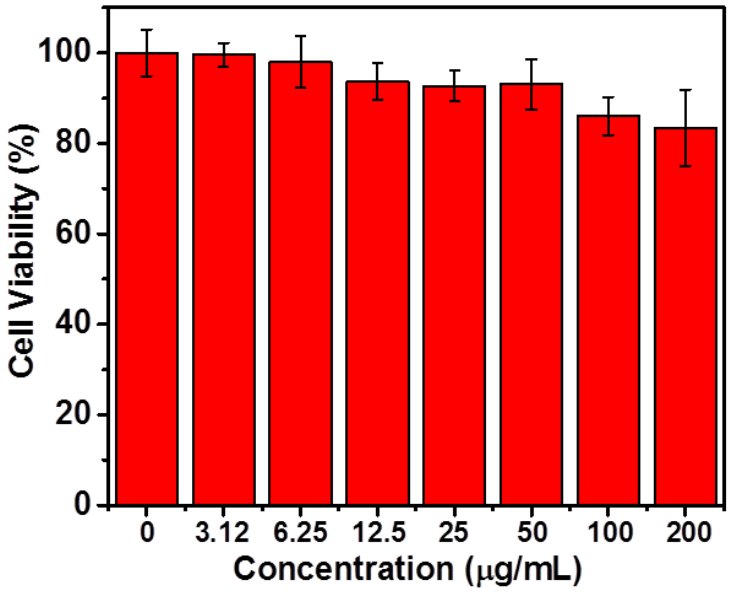

Fig. S17 In vitro cell viability test against L929 cells with different concentrations of CPA.

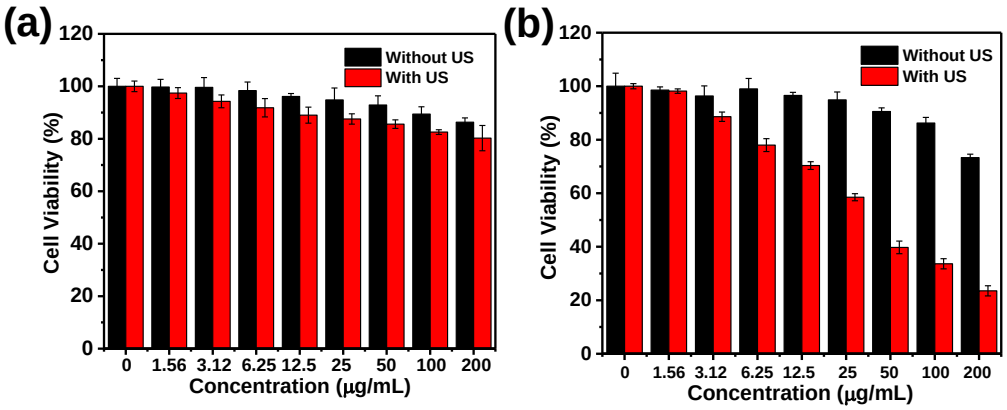

Fig. S18 In vitro cell viability test against 4T1 cells with different concentrations of (a) COF and (b) CPA with or without US irradiation.

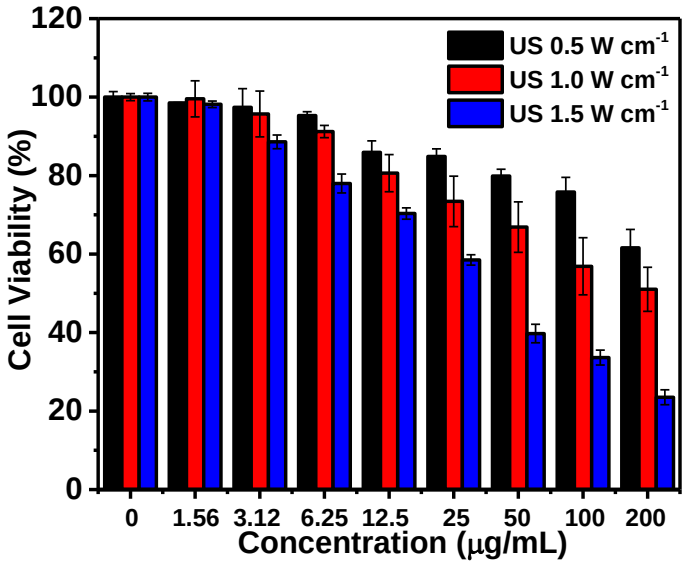

Fig. S19 In vitro cell viability test against 4T1 cells with different intensity of US.