Electronic Supplementary Information

DHA/Fe^{2+}-doped porphyrin COFs enable ROS-amplified multimodal tumor therapy†

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1. Materials and methods

1.1 Materials and instruments.

Materials. $FeCl_2 \cdot 4H_2O$ was commercially supplied by China National Pharmaceutical Group Corporation (Shanghai, China). We prepared 1,3,5,7-Tetrakis(4-aminophenyl) adamantane (TAPA) and 5,10,15,20-tetrakis(4-benzaldehyde) porphyrin (TBAP) according to previously reported methods. Dihydroartemisinin (DHA) and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Tokyo Chemical Industry Co., Ltd. (Japan) and Sigma Chemical Company, respectively. Gibco (USA) provided the Fetal bovine serum (FBS) and Nanjing KeyGen Biotech Co., Ltd. (China) provided RPMI 1640 medium. 2',7'-dichlorofluorescin diacetate (DCFH-DA) and the Calcein-AM/PI Double Stain Kit were purchased from Beyotime (Nantong, China). We obtained 96-well plates from Hangzhou Xinyou Biotechnology and 4T1 cells from Procell Life Science & Technology (both in China). Milli-Q purified water (resistivity 18.2 $M\Omega \cdot cm^{-1}$) was used in all preparations. Commercially available analytical-grade chemicals were used without additional purification.

Instruments. Nicolet iS50 spectrometer was used to analyze the chemical structures of the Fourier-transform infrared (FT-IR) spectroscopy. JEM-100CX II electron microscope (JEOL, Japan) was used to obtain the transmission electron microscopy (TEM) images. UV-vis were recorded on the Shimadzu UV-1700 spectrophotometer (Japan). The concentration of iron (Fe) was determined by inductively coupled plasma atomic emission spectroscopy (ICP-AES, iCAP 7400, Thermo Fisher). The progression of each reaction step was verified by tracking zeta potential shifts on the Malvern Zetasizer Nano (Malvern Instruments, UK). Absorbance readings were taken with a Synergy 2 microplate reader (BioTek, USA) for the MTT assay. Confocal fluorescence imaging was acquired by the Leica TCS SP8 laser scanning microscope (Germany).

1.2 Preparation of nanoscale COF

TAPA (13 mg, 0.026 mmol), TBAP (22.8 mg, 0.031 mmol), 1.0 mL of 1,2-dichlorobenzene, and 0.2 mL of 6 M acetic acid (aqueous solution) were put into an ampule (10 mm o.d., 8 mm i.d.). Then it was immersed in liquid nitrogen (77 K) for flash-freezing, evacuated to below 8 Pa, and flame-sealed under vacuum. Subsequently, the reaction mixture was heated to 140 °C for 5 days, which produced a yellow solid. The product was isolated by filtration, sequentially washed with anhydrous N,N-dimethylacetamide and anhydrous tetrahydrofuran (THF), and subsequently soaked in anhydrous THF for 24 hours. Finally, the solid was dried at ambient temperature followed by vacuum drying at 100 °C overnight[1]. In order to obtain nanoscale COF, the as-synthesized COF was ground with an agate mortar for 2 hours. The sample was then subjected to ultrasonic vibration treatment using an ultrasonic oscillator for one hour at least. Following centrifugation at 13,000 rpm for 10 minutes, the nanoscale COF was retrieved for further applications.

1.3 Preparation of COF(Fe)

4 mg nanoscale COF and 28.4 mg $FeCl_2 \cdot 4H_2O$ were dispersed in 4mL dimethylformamide (DMF). The mixtures were then subjected to reflux under an argon atmosphere for 4 hours at 90 ℃. Subsequently centrifugation at 14,500 rpm and successive washing with DMF and methanol, COF(Fe) was obtained. The iron concentration in the collected supernatant was subsequently determined by ICP-MS.

1.4 Preparation of COF(DHA) and COF(Fe)-DHA

The COF(Fe) (1.0 mg) was dispersed in distilled water (1 mL). Then DHA (4.0 mg) dissolving in 0.5 mL ethanol-water mixture (ethanol/water, v/v=3:1) was introduced and magnetically stirred at room temperature for 48 hours. After centrifugation at 14,500 rpm, deionized water was used to wash the precipitate for 2 times. The collected supernatant was analyzed to determine the drug loading efficiency based on standard calibration curves. COF(DHA) was prepared by the same procedure except COF(Fe) were displaced by COF. After centrifugation at 14,500 rpm,

the precipitate was washed twice with deionized water. The collected supernatant was analyzed to determine the drug loading efficiency based on standard calibration curves. COF(DHA) was synthesized following an identical procedure, with COF being used in place of COF(Fe).

1.5 Theoretical calculations

The calculations of Fenton and Fenton-like reactions were performed with Gaussian 09[2]. Geometry optimizations and frequency analyses were performed in aqueous solution at the PBE0-D3 functional and 6-31+G(d) basis set, with the solvent described by the CPCM model[3,4]. Each optimized structure was confirmed as a true minimum (no imaginary frequencies) or transition state (one imaginary frequency). Quintet state (S =2) has been confirmed ground state of this system[5].

1.6 Singlet oxygen ($^1O_2$) detection

The singlet oxygen ($^1O_2$) generation capabilities of COF and COF(Fe)-DHA were detected by DPBF as a probe. The degradation of DPBF (0.5 mM) was monitored in a solution containing 100 μg/mL of COF or COF(Fe)-DHA. Following irradiation with a 635 nm (0.2 W/cm^2) irradiation for various times, the mixture's supernatant was analyzed by UV/vis spectroscopy.

1.7 Detection of hydroxyl radicals

The hydroxyl radical (·OH) generation was detected by methylene blue (MB) and 3,3',5,5'-tetramethylbenzidine (TMB) as a probe. MB or TMB was added to solutions of COF or COF(Fe) (0.5 mg/mL) with or without of H_2O_2. Then the mixtures were incubated for 60 minutes, and they were centrifuged, lastly the supernatants were recorded by UV-vis absorption spectra.

1.8 Total ROS detection

DCFH was used to evaluate the ROS generation effects of COF, COF(Fe), COF(DHA) and the COF(Fe)-DHA (100 μg/mL). The solutions were respectively supplemented with DCFH and then irradiated with 635 nm laser for 5minutes. The fluorescence spectra of the solutions with various materials were measured using fluorescence spectroscopy. Ex= 488 nm, Em=525 nm. The same method was employed to measure the ROS production activity of different COF(Fe) rations.

1.9 Cell culture

4T1 cells were cultured in RPMI 1640 medium containing 10% FBS and 100 U mL^{-1} of 1% antibiotics (penicillin/streptomycin) and were maintained at 37 °C in a 5% CO_2/95% air humidified incubator (SANYO).

1.10 Live/dead cell staining

4T1 cells were seeded onto confocal dishes and cultured over nigh, then split into 5 groups: (I) PBS+L, (II) COF+L, (III) COF(Fe)+L, (IV) COF(DHA)+L, (V) COF(Fe)-DHA+L to observe the viable and dead cells. The concentration of nanoparticles was 0.1mg/mL. Laser irradiation groups were irradiated with a 635nm laser for 5minutes and then further cultured with other group for 12 hours. Cells were then incubated with Calcein AM (λex=490 nm, λem=515 nm) and Propidium Iodide (PI) (λex=535 nm, λem=617 nm) for 15 minutes prior to CLSM imaging

1.11 Intracellular ROS detection

Dichlorofluorescein diacetate (DCFH-DA) was used as a fluorescent probe to evaluate cellular ROS content. In brief, 4T1 cells were seeded onto confocal dishes and cultured. 12 hours later, after removing the culture medium, the cells were incubated with fresh media containing nanoparticles (100 μg mL^{-1}) for 6 hours. After that, cells were incubated with 10% v/v (100 μM) DCFH-DA in PBS for 15 minutes at 37 ℃. Following treatment, samples were rinsed with PBS and immediately imaged for green fluorescence using a CLSM. λex = 488 nm, λem = 500-550 nm.

1.12 Cell viability study

To evaluate the inhibitory effects of the different materials on cells, cell viability was quantified using the MTT assay. After seeding in 96-well plates and culturing for a period of time, 4T1 cells were assigned to five groups (PBS, COF, COF(Fe), COF(DHA), and COF(Fe)-DHA) and respectively treated with or without laser irradiation. Following incubation with nanoparticles (0.1 mg/mL) at 37 °C for 6 hours, the solutions were removed and replaced with fresh culture medium. Subsequently the cells were irradiated with 635nm laser. The cells were then incubated for an additional 12 hours. Lastly, we removed the culture medium, each well was installed 200 μL MTT solution (0.5 mg/mL). After four hours incubation the solutions was removed, 200 μL dimethyl sulfoxide (DMSO) was installed to dissolve the formazan crystals, and the microplate reader was used to measure the absorbance at 490 nm.

1.13 Animals and Tumor Model

All animal experiments were conducted and agreed with the Principles of Laboratory Animal Care (People's Republic of China), the Guidelines of the Animal Investigation Committee, Biology Institute of Shandong Academy of Science, China and the Ethics Committee of Shandong Normal University, Jinan, P. R. China (approval number AEECSDNU 2021066). Mice (8 weeks old, female, ~20 g) were maintained on normal conditions on a 12 hours light/dark cycle with ad libitum access to standard chow and sterile water. 1×10^7 4T1 cells were injected subcutaneously into the right axillary region of the Balb/C mice. When reaching a tumor volume of approximately 75-100 mm^3, the mice were subsequently utilized for further experimentation.

1.14 In vivo therapeutic effect and biosafety evaluation

The calculation formula of tumor volume: V = (tumor width)^2 × (tumor length)/2. The tumor-bearing mice were divided into seven groups: (I) PBS, (II) PBS+L, (III) COF(Fe)-DHA, (IV) COF(Fe)+L, (V) COF(DHA)+L, (VI) COF(Fe)-DHA+L. Different materials (0.5 mg/mL) were injected into the primary tumor sites of mice. Then, mice were irradiated by 635 nm laser for 5 minutes. Body weights, volumes of primary and distal tumors were recorded every 2 days. After 14 days, the primary tumors and main organs were collected for H&E staining.

2. Supporting figures

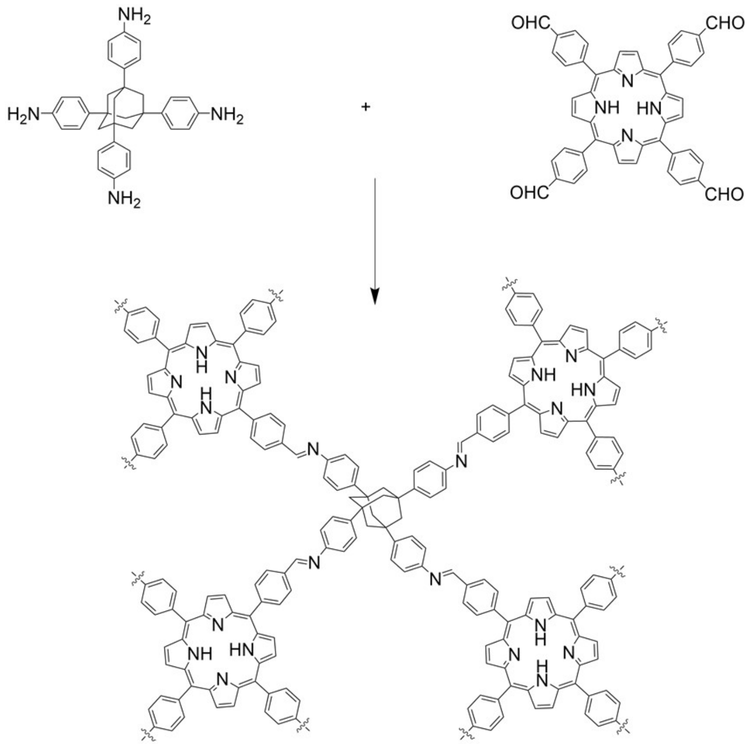

Fig. S1 Synthesis of porphyrin-based COF.

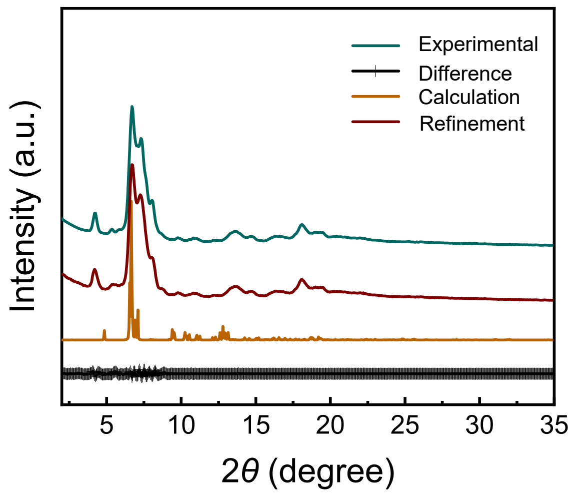

Fig. S2 The XRD pattern of porphyrin-based COF.

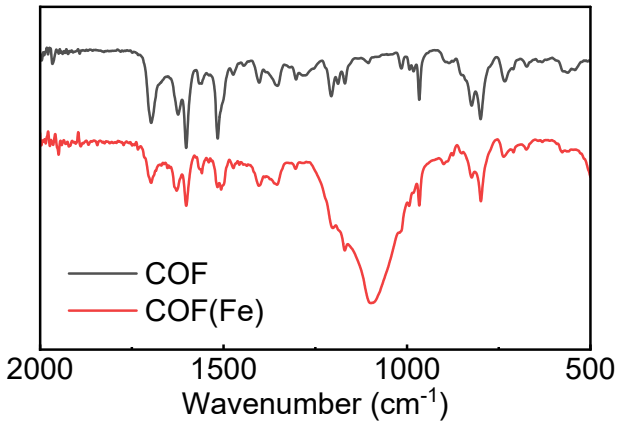

Fig S3. FT-IR spectrum of COF and COF(Fe).

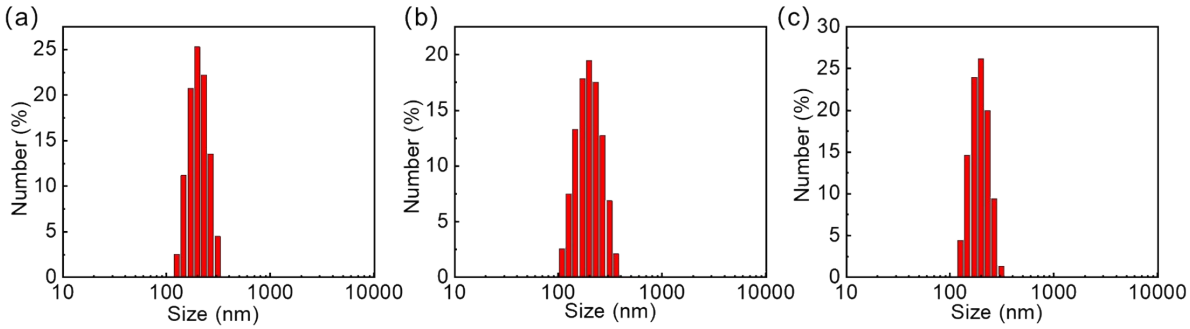

Fig S4. The size distribution of (a) COF, (b) COF(Fe) and (c) COF(Fe)-DHA.

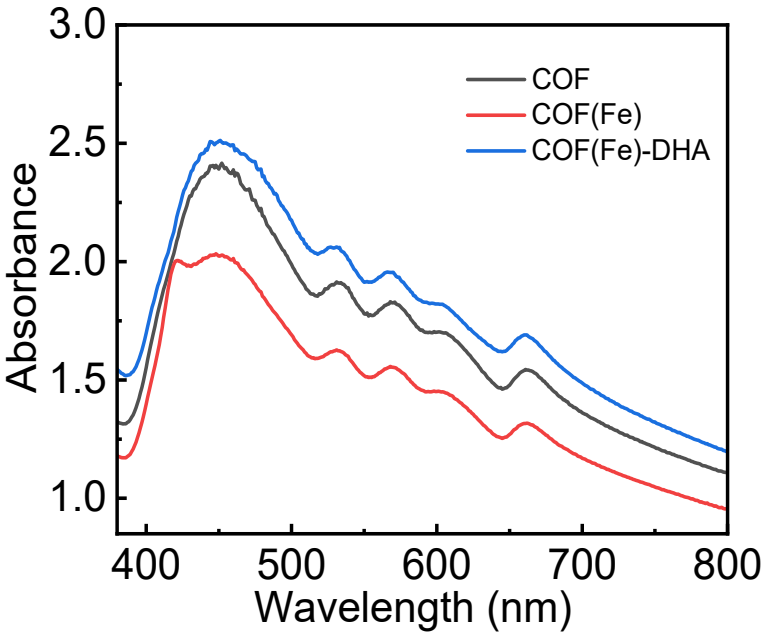

Fig. S5 The UV-vis spectra of COF, COF(Fe) and COF(Fe)-DHA nanoparticles.

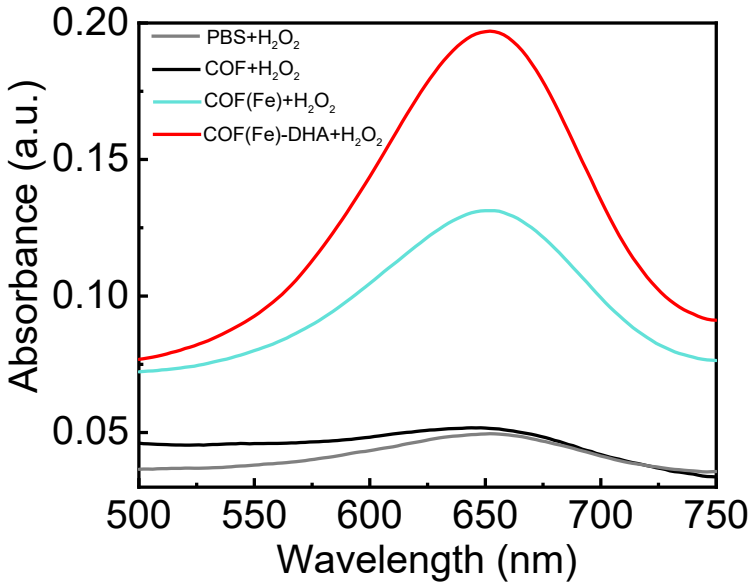

Fig. S6 UV–vis absorption spectra of TMB after incubation with COF, COF(Fe) and COF(Fe)-DHA.

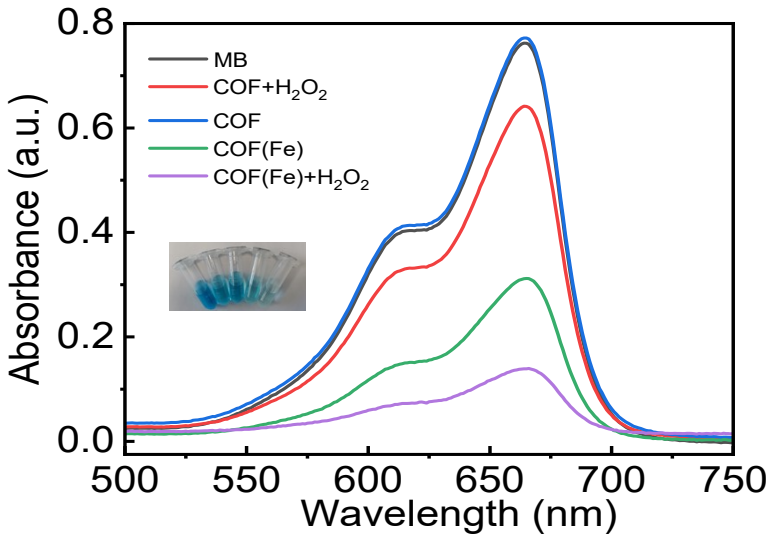

Fig. S7 The generation of hydroxyl radical (•OH) measure by MB degradation in COF or COF(Fe) with or without H_2O_2.

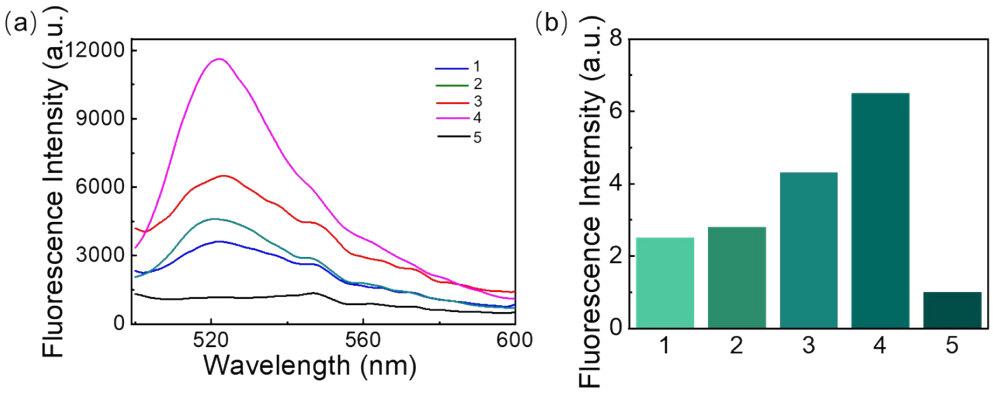

Fig. S8 (a)The fluorescence intensity of DCFH probe with different treatments. (b) Quantitative analysis of relative fluorescence intensity across experimental groups. COF: $Fe^{2+}$are (1) 1:0; (2) 4:1; (3) 1:1; (4) 1:2; (5) 1:4; respectively.

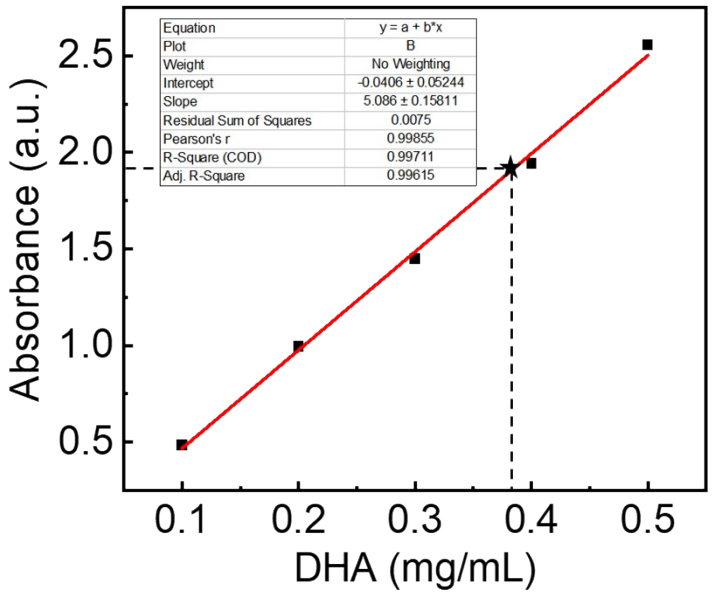

Fig. S9 The DHA standard curve UV-vis for absorbance values at 290 nm. The star-marked areas represent the concentrations in the supernatant solution.

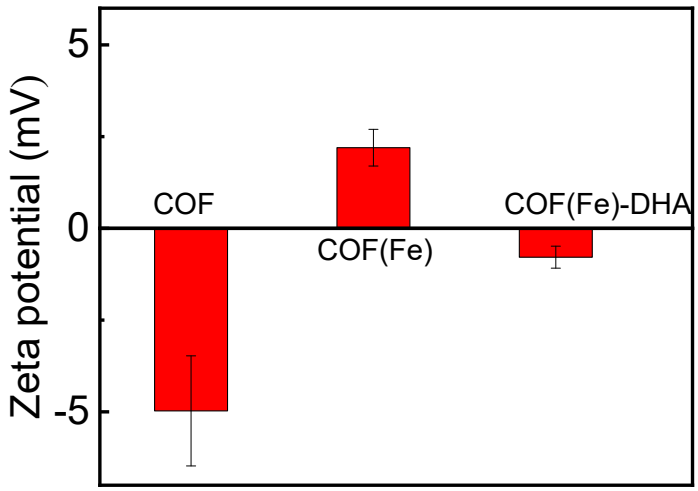

Fig. S10 Zeta potential of COF, COF(Fe) and COF(Fe)-DHA.

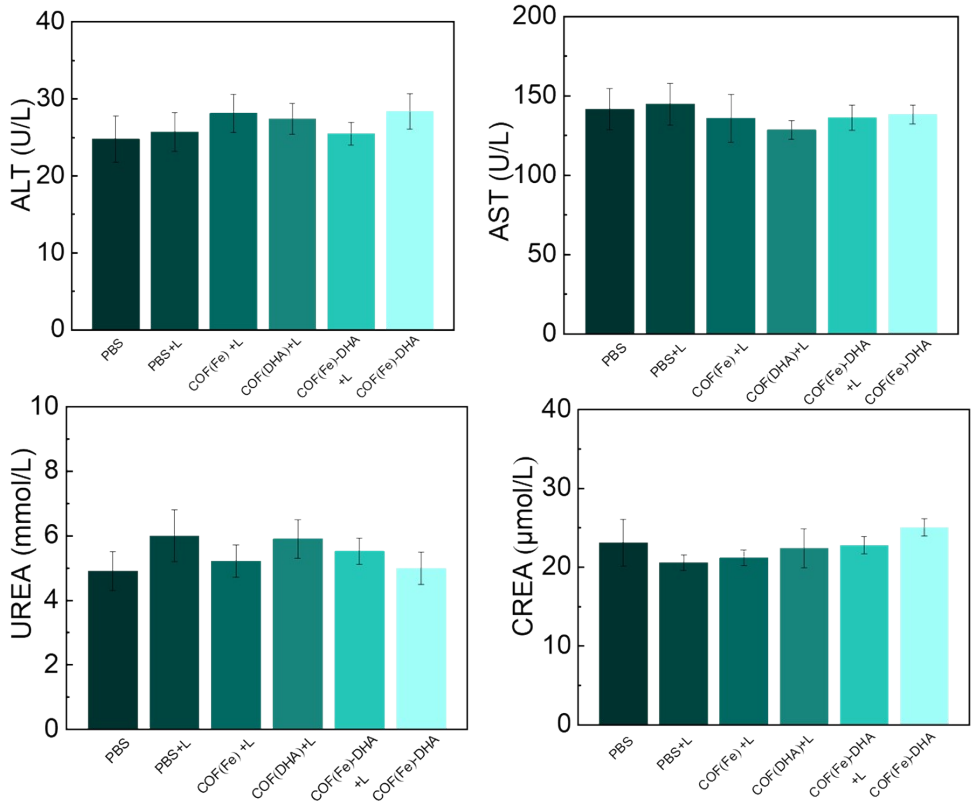

Fig. S11 (a) The results of serum biochemistry of mice intratumorally injected with different materials. PBS was employed as the control.

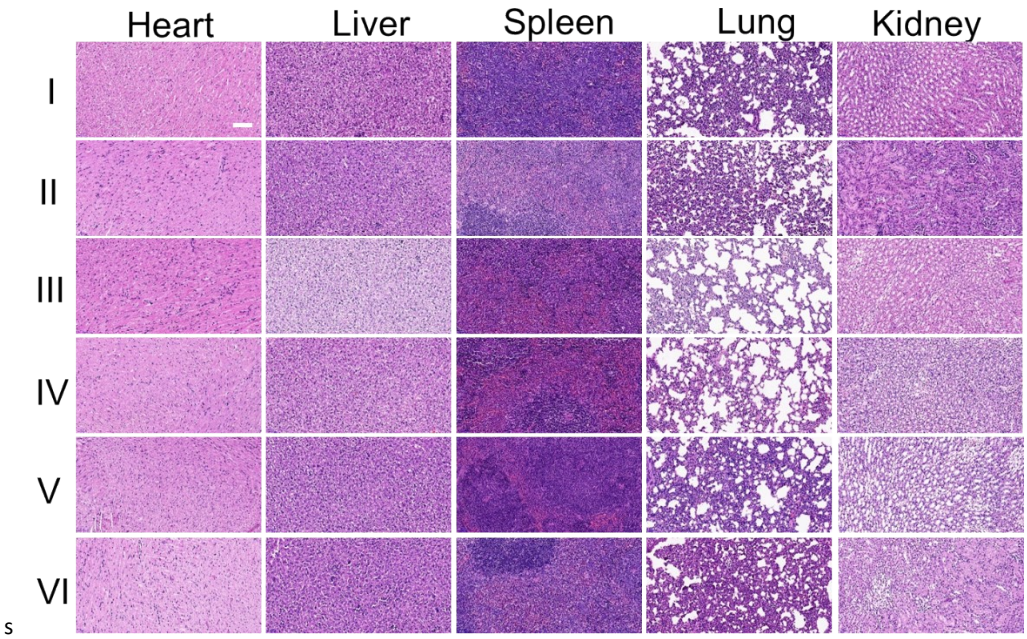

Fig. S12 H&E staining of main organs of mice in different groups. Scale bar is 100 μm, Magnification is 20×.

3. Supporting table

element	concentration (ppm)
Fe	3.963
Fe	4.270
Fe	4.552

Table S1 The concentration of Fe in supernation.

4. References

1. R. Liu, Z. Wen, B. Zheng, J. Yang, M. Wang, X. Ding, L. Shi, H. Hu, T. Chen, S. Xiao, Y. Gao, *Sep. Purif. Technol.*, 2025, **370**, 133234

2. M. Frisch, G. Trucks, H. Schlegel, G. Scuseria, M. Robb, J. Cheeseman, G. Scalmani, V. Barone, B. Mennucci, G. J. I. Petersson, Gaussian 09 (Revision A.02), Gaussian Inc., Wallingford, CT, 2009.

3. V. Barone, M. Cossi, *J. Phys. Chem. A,* 1998, **10**, 1995-2001.

4. M. Cossi, N. Rega, G. Scalmani, V. Barone, *J. Comput. Chem.* 2003, **24**, 669-681.

5. H. F. Lu, H. F. Chen, C. L. Kao, I. Chao, H. Y. Chen, *Phys. Chem. Chem. Phys.*, 2018, **20**, 22890-22901.