

## Supporting Information

### **Multifunctional Red Carbon Dots: A Theranostic Platform for Magnetic Resonance Imaging and Fluorescence Imaging-Guided Chemodynamic Therapy**

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## 1. Experimental Section

**Chemicals.** nitric acid ( $\text{HNO}_3$ ), hexane, DMSO and p-phenylenediamine (p-PD) were obtained from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China) Ethylenediaminetetraacetic acid (EDTA), Hydroxy-2,5-dioxopyrrolidine-3-sulfonicacid sodium salt (NHS, 97%), 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC, 99%),  $\text{GdCl}_3$  and  $\text{FeCl}_3 \cdot 4\text{H}_2\text{O}$  were purchased from shanghai Aladdin Company (Shanghai, China). Dulbecco's modified eagle medium (DMEM), Fetal bovine serum (FBS) and methyl thiazolyl tetrazolium (MTT) was obtained from SunShine Biotechnology Co., Ltd. (China). 2,7-Dichlorodi-hydrofluorescein diacetate (DCFH-DA), calcein AM, propidium iodide (PI), and A549 cells were purchased from the Jiangsu KeyGEN BioTECH Corp., Ltd (Nanjing, China).

**Synthesis of red carbon dots (CDs).** The CDs were prepared as described in the literature report<sup>1</sup>. Firstly, 0.1 mol/L p-PD aqueous solution was mixed with 2.5 mol/L  $\text{HNO}_3$  under stirring. Then, the mixture was sealed into a Teflon-lined autoclave and kept for reaction at 200 °C for 2 h, and then naturally cooled to room temperature. After centrifugation and washing by hexane for three times and drying in a vacuum oven at 60 °C, the CDs were obtained as the dried powder.

**Synthesis of CDs@EDTA.** The CDs@EDTA were synthesized as follows. The CDs powder (0.20 g) was dissolved into 20 mL DMSO, then 0.35 g EDTA, 0.35 g of EDC (1.82 mmol) and 0.20 g of NHS (0.91 mmol) was sequentially added into the solution under magnetic stirring and kept at ambient temperature for 24 h. Afterwards, 150 mL ethanol was added to get a suspension. The suspension was centrifuged and washed with ethanol for 3 times to get the CDs@EDTA.

**Synthesis of CDs@EDTA@Gd@Fe.** The CDs@EDTA@Gd@Fe were prepared by mixed EDTA with an equimolar concentration of  $\text{GdCl}_3$  and

FeCl<sub>3</sub>·4H<sub>2</sub>O aqueous solution for several hours. All steps were carried out under N<sub>2</sub> conditions to keep Fe<sup>2+</sup> from being oxidized. The obtained suspension were separated by centrifugation and washed with ethanol for 3 times. After that, the products were dried and kept in a vacuum condition.

**Characterization.** The morphology of samples was studied by transmission electron microscope (TEM, Tecnai G2 F30 S-TWIN). The size distribution of samples was measured through the dynamic light scattering (DLS, Malvern Nano-ZS 90 Nanosizer). Ultraviolet-visible (UV-vis) absorption spectra was recorded by a UV absorption spectrophotometer (Cary-50, varian, USA). The surface composition and element measurement of the samples was performed on X-ray photoelectron spectroscopy (XPS, ESCALAB 250, Thermo Fisher). Reactive oxygen species were measured through electron spin resonance (ESR) spectra (JEOL Ltd., Tokyo, Japan). *In vitro* fluorescence images were collected with a confocal laser scanning microscope (TI-EA1R, Nikon, Japan). The fluorescence spectra of samples was recorded by LS55 fluorescence spectrophotometer (PerkinElmer).

**Cytological experiment.** A549 cells were cultured under hypoxic environment (5% CO<sub>2</sub> at 37 °C) supplemented with DMEM containing with 10% FBS, 1% streptomycin, and 1% penicillin. Cells were seeded in 96 well plates at a density of 5×10<sup>3</sup> cells/well after for 24 h and co-cultured with PBS, H<sub>2</sub>O<sub>2</sub>, CDs@EDTA@Gd, CDs@EDTA@Gd@Fe (100 µg/mL) for another 24 h. Then, all cells were stained with calcein AM/PI and observed by a CLSM. Cell viability was determined by an MTT assay. Briefly, A549 cells at a density of 5×10<sup>3</sup> cells/well were seeded in 96-well plates. After culture for 24 h, the cells were treated with PBS, H<sub>2</sub>O<sub>2</sub>, CDs@EDTA@Gd, and CDs@EDTA@Gd@Fe. After further 24 h, the culture medium was replaced by fresh medium together with MTT reagent (5 mg/mL). After co-incubation for 4 h, MTT-containing

medium was removed and 150  $\mu\text{L}$  DMSO was added to all wells. A microplate reader was used to record the OD values at 570 nm of different experimental wells to analyze cell viability<sup>2</sup>.

**Study of hydroxyl radicals ( $\bullet\text{OH}$ ) generation.**  $\bullet\text{OH}$  produced on the sample was directly displayed by reactive Oxygen Species (ROS) fluorescence staining.  $1 \times 10^6$  cells were inoculated on the surface of samples at a density of  $60 \mu\text{L}/\text{cm}^2$ . After 6 h of culture, all samples were stained with DCFH-DA in dark for 30 min, and then rinsed with normal saline and observed under fluorescence microscope.

**Hemolysis and the shape of RBCs.** RBCs was acquired by the centrifugation of 5 mL whole blood with anticoagulation at 3000 rpm for 15 min, and then diluted with PBS ( $V_{\text{PBS}}:V_{\text{RBCs}} = 9:1$ ). Afterward, 0.5 mL of RBCs suspension was incubated with different concentrations of CDs@EDTA@Gd@Fe (2 mL in PBS). 0.5 mL of RBCs suspension was mixed with 2 mL of deionized water and PBS as positive and negative controls, respectively. After incubated at  $37^\circ\text{C}$  for 3 h, the mixtures were centrifuged at 3000 rpm for 15 min to collect supernate. A microplate reader (BioTek synergy 2) was employed to measure the optical density (OD) of the supernatant at 541 nm. The hemolysis rate was calculated according to the following formula<sup>3</sup>:

$$\text{Hemolysis rate\%} = (\text{OD}_{\text{sample}} - \text{OD}_{\text{negative}}) / (\text{OD}_{\text{positive}} - \text{OD}_{\text{negative}}) \times 100\%$$

where  $\text{OD}_{\text{sample}}$ ,  $\text{OD}_{\text{positive}}$ , and  $\text{OD}_{\text{negative}}$  were the OD of the sample, positive control, and negative control, respectively. The RBCs shape was observed by a light microscopy (BM2100, Nanjing Jiangnan Novel Optics Co., Ltd).

**Animal model.** Female nude mice (5-7 weeks old) were obtained from Jiangsu KeyGEN BioTECH Corp., Ltd. All performance of *in vivo* experiments were in

line with the institutional animal use and care regulations approved by of Nanjing Normal University. The tumor-bearing mice model was developed by subcutaneously injection with A549 cells.

***In vivo MRI/FL imaging.*** The intravenous injection of CDs@EDTA@Gd@Fe (100  $\mu$ L, 10 mg/kg) in tumor-bearing mice which were used to perform the *in vivo* MRI and FL. The MRI photos were obtained through Bruker Icon 1.0 T scanning mice at different time points. The FL images were collected by UVP iBOX Scientia 900 at different time points.

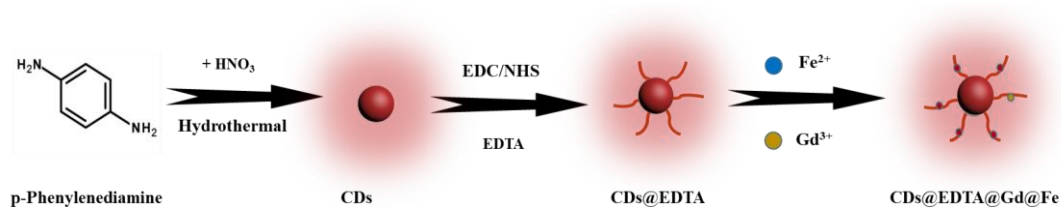
***Anti-cancer performance of CDT in vivo.*** When the volume of tumors reached  $\sim 60 \text{ mm}^3$ , *in vivo* CDT were conducted in four groups (n = 5 per group) after intravenous injection with PBS,  $\text{H}_2\text{O}_2$ , CDs@EDTA@Gd, and CDs@EDTA@Gd@Fe, respectively. The changes of body weight and tumor volume were monitored every other day. The tumor volumes were calculated according to equation<sup>4</sup>  $V = \text{width}^2 \times \text{length}/2$  and the initial volume ( $V_0$ ) of the tumor were normalized to obtain the relative tumor volume ( $V/V_0$ ). After 14 days, the tumor tissue sections were collected for H&E staining.

***In vivo biodistribution.*** The biodistribution of CDs@EDTA@Gd@Fe in A549-bearing tumor nude mice were test *via* intraperitoneal injection of samples (10.0 mg/kg). At different times, the mice were euthanized, and the main organs, i.e., the heart, liver, spleen, lungs, and kidneys, and the tumor tissue were extracted and weighed. For the inductively coupled plasma atomic emission spectroscopy (ICP-AES) measurements, these tissues were subsequently cut into 1-2  $\text{mm}^2$  pieces and digested in aqua solutions for 1 day to detect Fe content.

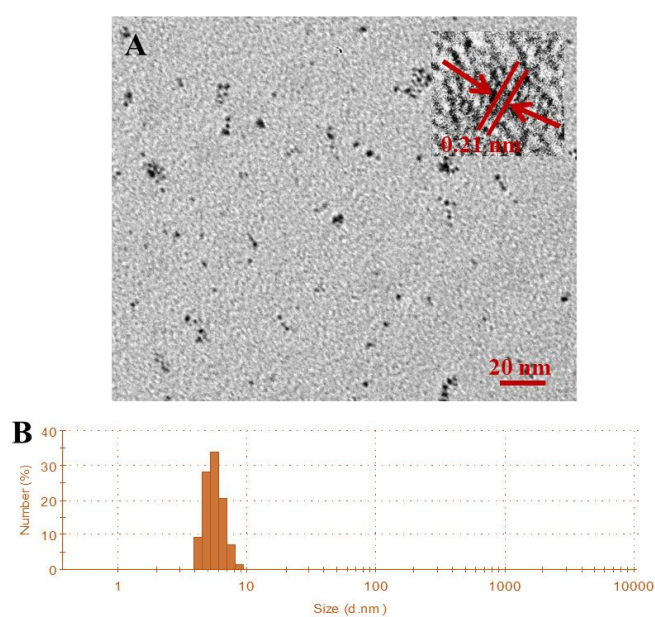
***In vivo toxicity assay.*** To evaluate the potential *in vivo* toxicity, blood biochemistry analysis was conducted. Blood was collected from mice after

injection for 1, 7, and 14 days, and the serum biochemistry was determined by using the blood biochemistry analysis kits (JCBIO, China). Blood cell counts were tested using an automated blood cell counter (BC-2800 Vet Analyzers, China).

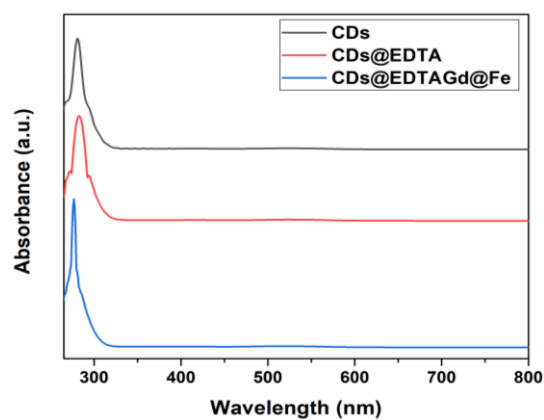
## 2. Supplementary Figures



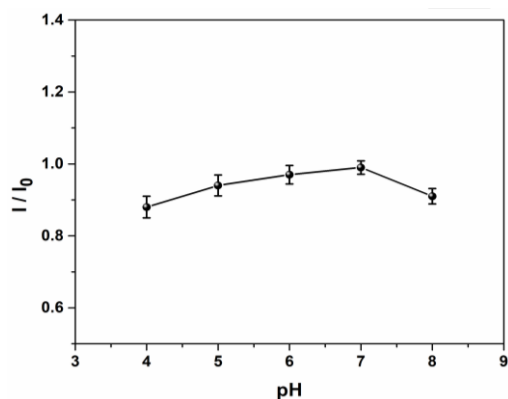
**Fig. S1** Schematic of the preparation of CDs@EDTA@Gd@Fe.



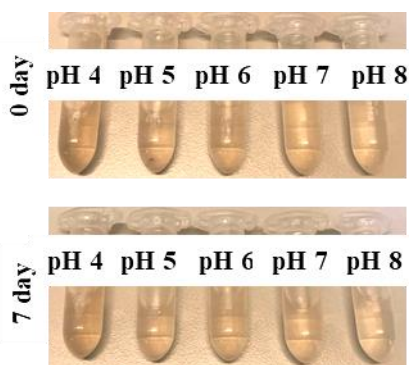
**Fig. S2** TEM image (A) and size distribution (B) of CDs.



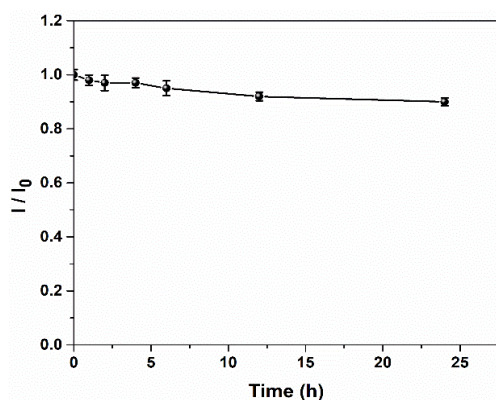
**Fig. S3** UV-vis absorption spectra of CDs, CD@EDTA, and CDs@EDTA@Gd@Fe.



**Fig. S4** Fluorescence response of CDs@EDTA@Gd@Fe in the different pH PBS solutions, ( $I_0$  and  $I$  correspond to the fluorescence intensity of the original CDs@EDTA@Gd@Fe and different pH solutions, respectively.).

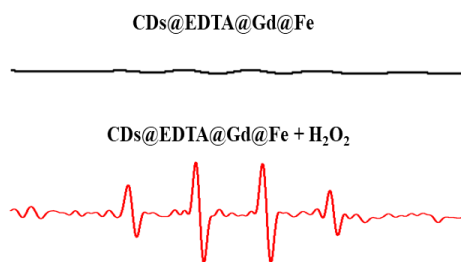


**Fig. S5** Photos of CDs@EDTA@Gd@Fe in the PBS solutions (different pH) after 7 days.

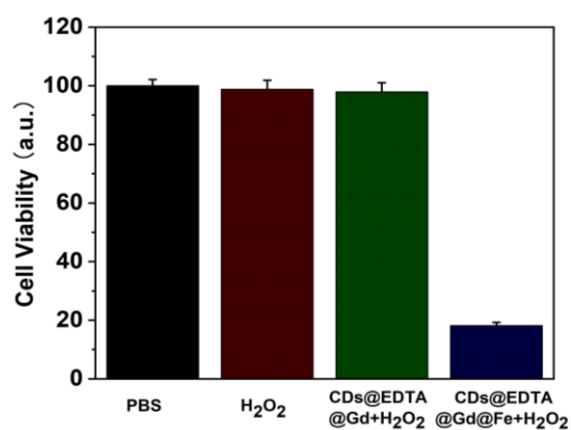


**Fig. S6** Fluorescence response of CDs@EDTA@Gd@Fe under a UV lamp (365 nm).

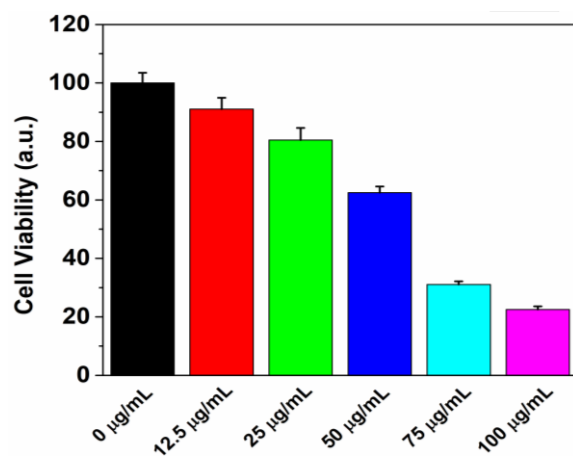




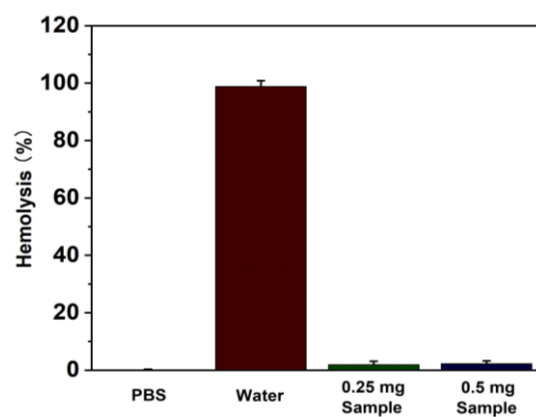
**Fig. S7** EPR spectra of CDs@EDTA@Gd@Fe with the DMPO as the spin trap with or without H<sub>2</sub>O<sub>2</sub> solution.



**Fig. S8** Cell viability of A549 cells of different treatment groups.



**Fig. S9** Cytotoxicity evaluation of CDs@EDTA@Gd@Fe towards A549 cells after at different concentrations.



**Fig. S10** Hemolysis of different treatment groups.

### 3. References

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2. C. Zhao, L. Shao, J. Lu, X. Deng, Y. Tong and Y. Wu, *ACS Applied Materials & Interfaces*, 2017, **9**, 18450-18461.
3. F. Wu, M. Zhang, X. Chu, Q. Zhang, Y. Su, B. Sun, T. Lu, N. Zhou, J. Zhang, J. Wang and X. Yi, *Chemical Engineering Journal*, 2019, **370**, 387-399.
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