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A Nanobattery of Reactive Oxygen Species through Cascade Photo-chemodynamic Reaction for Enhanced Tumor Therapy

A-Min Cao,[‡] Wen-Long Liu,[‡*] Mei-Zhen Zou, Si-Yong Qin, Yin-Jia Cheng, Ai-Qing Zhang[*]

a. Key Laboratory of Catalysis and Energy Materials Chemistry of Ministry of Education & Hubei Key Laboratory of Catalysis and Materials Science, School of Chemistry and Materials Science, South-Central Minzu University, Wuhan 430074, P.R. China. b. Department of Clinical Laboratory, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, 430022, China.

‡ These authors contributed equally to this work.

Corresponding authors: wenlong@whu.edu.cn (Wen-Long Liu);

aizhang@scuec.edu.cn (Ai-Qing Zhang)

Experimental section

Synthesis of Fe_3O_4 nanoparticles

Citric acid modified Fe_3O_4 nanoparticles were synthesized via a Massart precipitation method.[1] Briefly, $FeCl_2$ (0.43 g) and $FeCl_3$ (1.18 g) were dissolved in 20.0 mL of deionized water. And then 3.0 mL of NH_3-H_2O (25~28%) was introduced into the above solution with stirring for 30 min at 80 ℃ under argon atmosphere. The reaction was maintained for 1.5 h at 95 ℃ after the addition of citric acid solution (0.25 g mL^{-1}, 2mL). This reaction solution was cooled down to room temperature with stirring and then the unreacted raw materials were removed by dialyzed (10 KD cut-off molecular weight) against deionized water.

Separation of red blood cell membrane

The blood was collected with ethylene diamine tetraacetic acid (EDTA) for anti-freezing from the healthy Babl/c mice. Erythrocytes were obtained by centrifugation with rotate speed of 3000 rpm for 5 min and washed with cold phosphate buffered saline (PBS, pH 7.4) for thrice. Then the obtained red blood cells were dispersed in deionized water (as hypotonic solution) to lead the cells burst. The solution of cell debris was centrifugated with rotate speed of 14000 g for 30 min to get red blood cell membrane by separating. Repeat the procedure of hypotonic rupture and centrifugation until the supernatant was colorless. The obtained red blood cell membrane was lyophilized and stored at -80 °C for further use.[2]

Decoration of red blood cell membrane

The lyophilized red blood cell membrane was rehydrated in PBS. Under ultrasound processing, docosahexaenoic acid (DHA) was mixed with cell membrane solution with the weight ratio (red blood cell membrane versus DHA) of 2:1 to obtain M(DHA). Then, a photosensitizer Ce6 was immobilizated onto M(DHA) by amidation to prepared M(DHA)-Ce6.[3] Specifically, 0.1 mg of Ce6, 0.098 mg of 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) and 0.07 mg of N-Hydro xysuc cinimide (NHS) were dissolved in 150 μL of DMSO with stirring for 1 h in dark. And then the mixed solution was dripped into M(DHA) solution (2 mg·mL^{-1}, 0.5 mL) and the reaction was kept overnight.

Preparation of Fe_3O_4@M(DHA)-Ce6

A successive physical extrusion process was done to obtain M(DHA)-Ce6 coating Fe_3O_4 (Fe_3O_4@RBCM(DHA)-Ce6).[4] The Fe_3O_4 nanoparticles solution was mixed with M(DHA)-Ce6 solution at the weight ratio of 1:1 with ultrasound treatment. Then the mixed solution was subjected to extruded through polycarbonate membranes with aperture of 800 nm and 450 nm, respectively. After that, the solution was magnetic treatment to remove the free red blood cell membrane and Ce6.

Cell culture

Murine colon cancer cells (CT26) were cultured with RPMI-1640 medium containing 10% fetal bovine serum (FBS) and 1%-1% penicillin-streptomycin at 37 °C in a humidified atmosphere containing 5% CO_2.

Detection of ROS in vitro

The ROS production in cells was evaluated using 2',7'-dichlorofluorescin diacetate (DCFH-DA) as ROS probe.[5] CT26 cells were seeded in confocal dishes and incubated for 24 h. Fe_3O_4@M(DHA)-Ce6, Fe_3O_4@M(DHA) and Fe_3O_4@M-Ce6 in RMPI 1640 media were added into the dishes, respectively. After incubating for 4 h, DCFH-DA (5 μM) was added into the dishes and incubated for 30 min. Then, the media were replaced with PBS and the cells were treated with or without light irradiation for 30 s (660 nm laser, 100 mW cm^{-2}) before CLSM observing.

Cytotoxicity assay

The cytotoxicity assay of Fe_3O_4@RBCM(DHA)-Ce6 to CT26 cells was conducted by MTT method.[6] Briefly, about 5×10^3 CT26 cells were seeded in 96-well plates. After 24 h incubation, Fe_3O_4@M(DHA), Fe_3O_4@M-Ce6 and Fe_3O_4@M(DHA)-Ce6 were added to each well, respectively. The cells were treated with or without light irradiated for 30 s (660 nm laser, 100 mW cm^{-2}) after 4h coincubation. Then, the cells were cultured for further 20 h. Next, MTT (5.0 mg mL^{-1}, 20 μL) was added to each well for another 4 h incubation. After that, the media were replaced with 150 μL of DMSO to resolved the purple products. The optical density (OD) at 570 nm of the purple solutions were measured using a microplate reader. The relative cell viability was calculated as following:

Cell viability (%) = (OD_s /OD_b× 100%

Where ODs represented the OD value of sample group, and OD_b represented the OD value of blank control at 570 nm.

The living and death cells staining assay against CT26 cells was also designed to display the cytotoxicity using the calcein-AM as probe of living cells and propidium iodide as probe of death cells. Briefly, CT26 cells were incubated with Fe_3O_4@M(DHA), Fe_3O_4@M-Ce6 and Fe_3O_4@M(DHA)-Ce6 for 4 h, respectively. Then, the cells were light irradiated with 660 nm laser for 30 s (100 mW cm^{-2}). Next, the media were replaced with PBS before the cells were stained with Calcein-AM and propidium iodide for 30 min. Then, the stained cells were washed with PBS to remove the extra dyes before CLSM observation.

Antitumor effect in vivo

Female Balb/c mice (5-weeks old, 20 g) were purchased from Animal Bio-safety level 3 Laboratory of Huazhong

Agricultural University (Certificate SCXK 2021-0021; Hubei, China). Animal use was approved by the Animal Ethics Committee of South-Central University for Nationalities (SYXK (Wuhan) 2016-0089, No. 2021-scuec-030). CT26 cells were inoculated subcutaneous of Balb/c mice. Once the tumors reached about 100 mm^3, the mice were administered with PBS, Fe_3O_4@RBCM(DHA), Fe_3O_4@RBCM-Ce6 and Fe_3O_4@RBCM(DHA)-Ce6 via the tail vein injection, respectively. On alternate days, the tumor volumes and body weight of mice were recorded. The tumor volume was calculated according to the formula: $V = L \times W^2 / 2$, where L was the long axis of the tumor and W was the short axis of tumor. After 21 d postinjection, the mice were mercifully killed and the major organs were collected. Tissue sections of these organs were stained with hematoxylin and eosin to observe the pathological state.

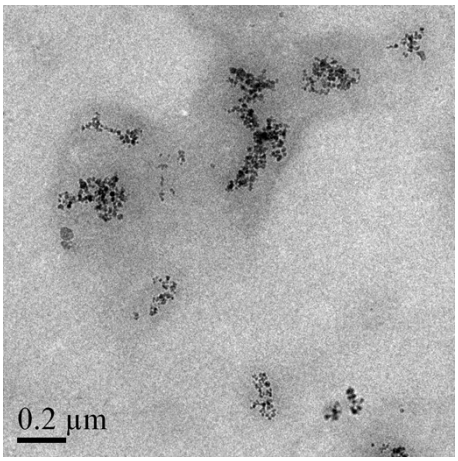

Fig. S1. TEM image of Fe_3O_4@M(DHA)-Ce6.

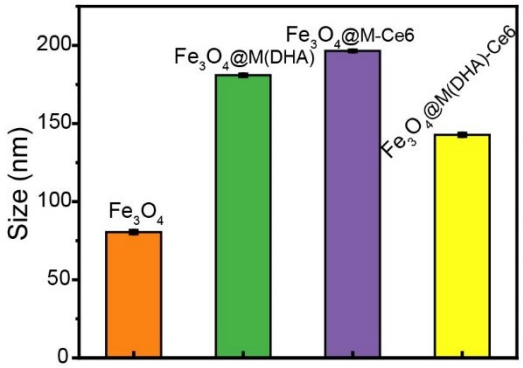

Fig. S2. Mean hydrodynamic diameter of Fe_3O_4, Fe_3O_4@M(DHA), Fe_3O_4@M-Ce6, and Fe_3O_4@M(DHA)-Ce6.

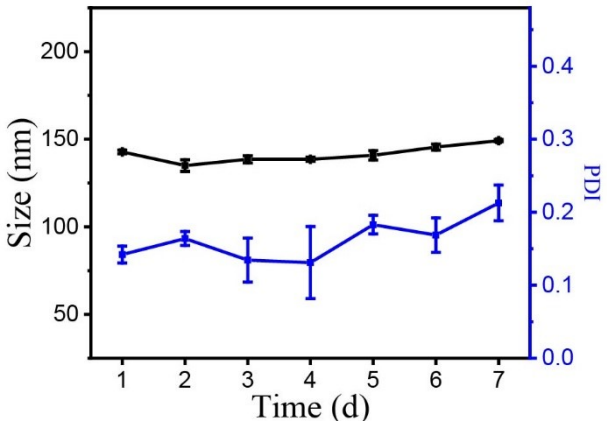

Fig. S3. The changes of hydrodynamic diameter of Fe_3O_4@M(DHA)-Ce6 with time in water.

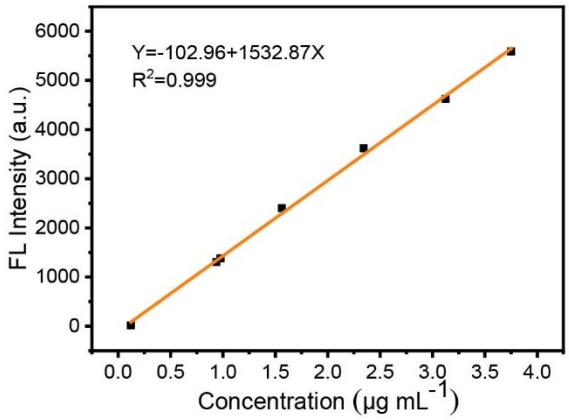

Fig. S4. The linear relationship between the Uv-vis absorbance and the Ce6 concentration.

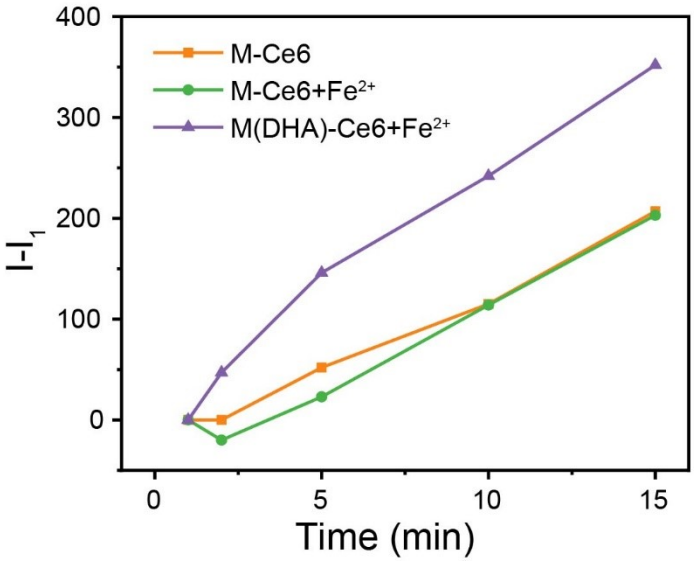

Fig. S5. The fluorescence intensity changes of M-Ce6 and M(DHA)-Ce6 after adding Fe^{2+}.

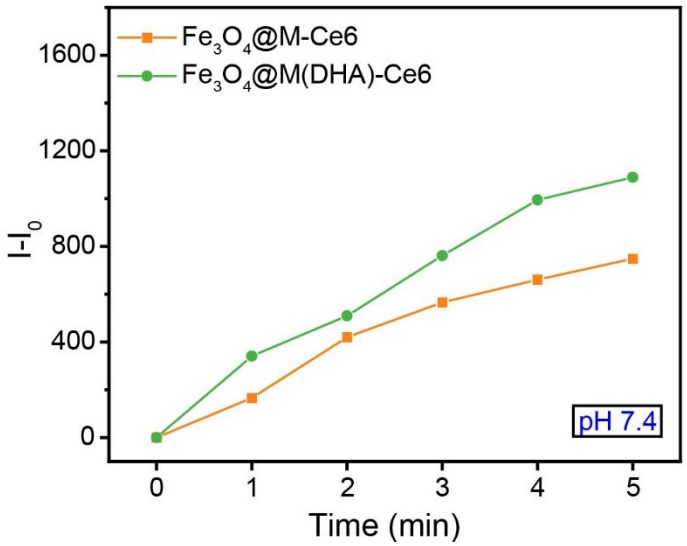

Fig. S6. The ROS production ability of Fe_3O_4@M-Ce6 and Fe_3O_4@M(DHA)-Ce6 with light irradiation at pH 7.4.

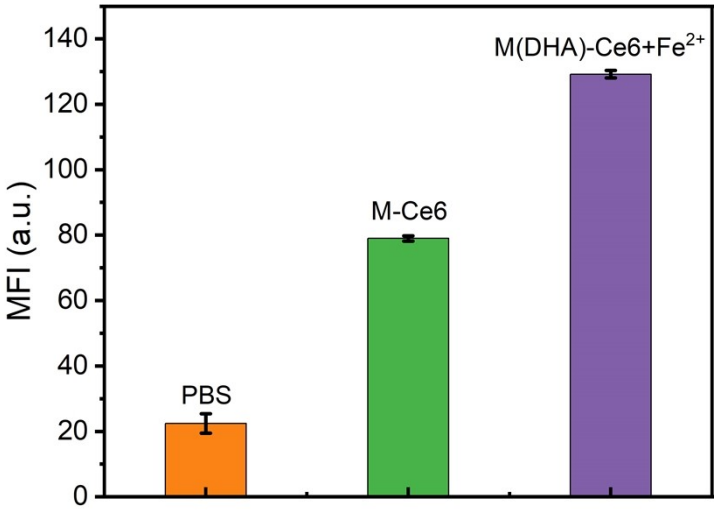

Fig. S7. The corresponding mean fluorescence intensity (MFI) in the cells with different treatments.

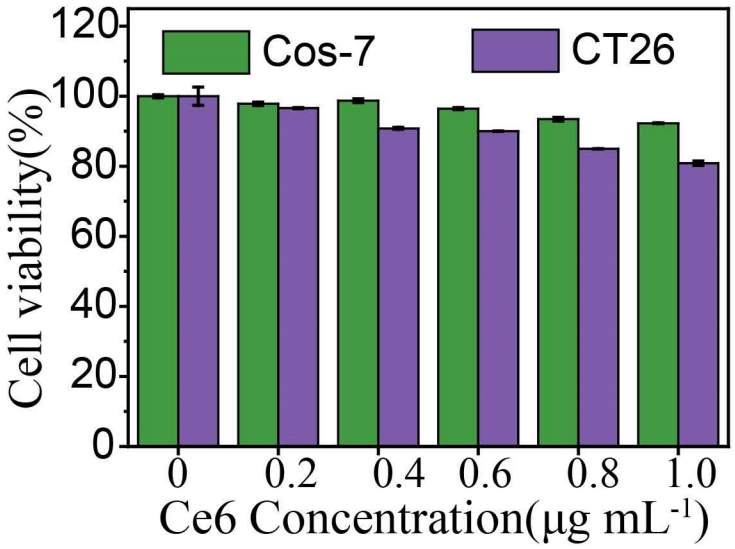

Fig. S8. The cytotoxicity of irradiation-treated Fe_3O_4@M(DHA)-Ce6 to Cos-7 and CT26.

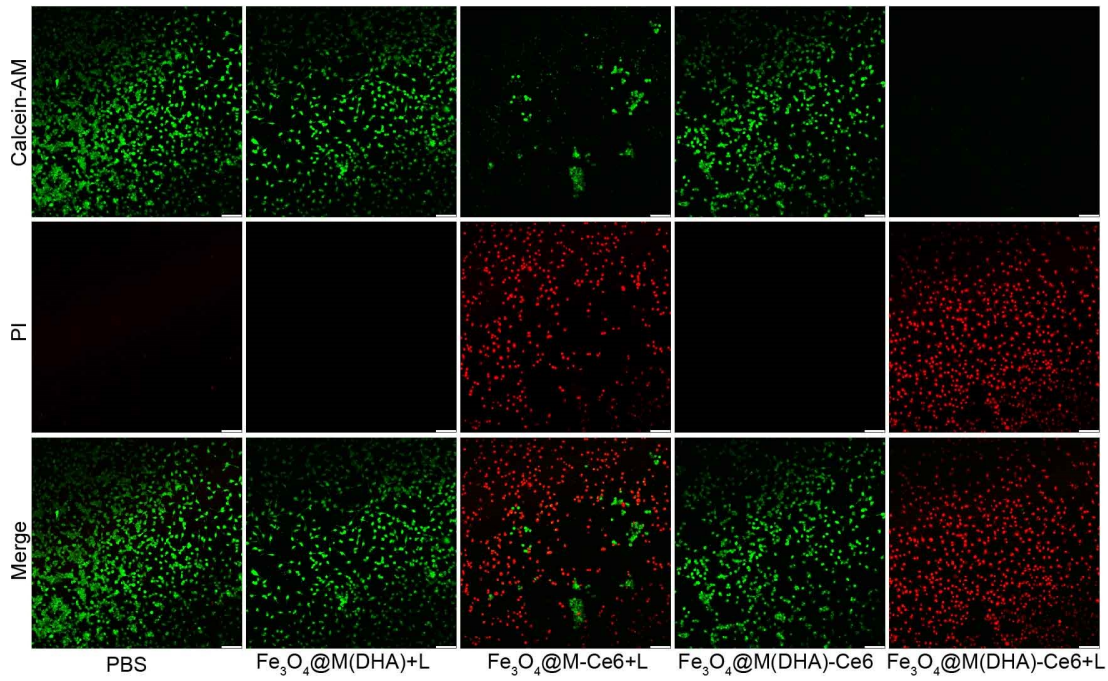

Fig. S9. Living and death cell staining images of CT26 cells after various treatment.

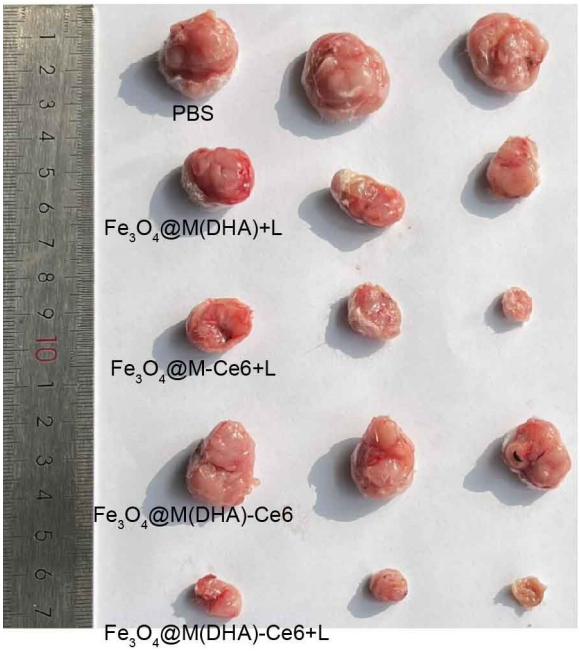

Fig. S10. The photos of tumors harvested after the end of the experiment.

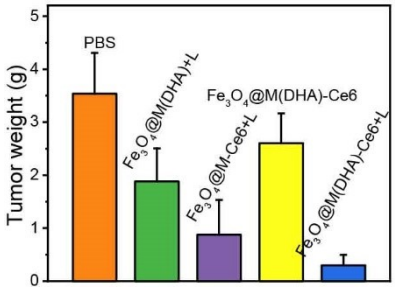

Fig. S11. The tumor weight after various treatments.

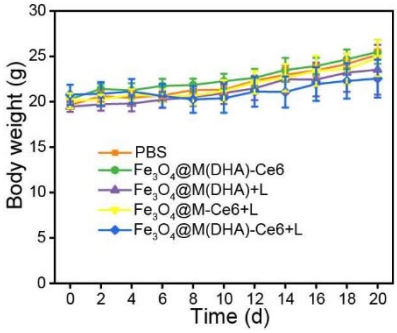

Fig. S12. The changes of body weight of mice after different treatments.

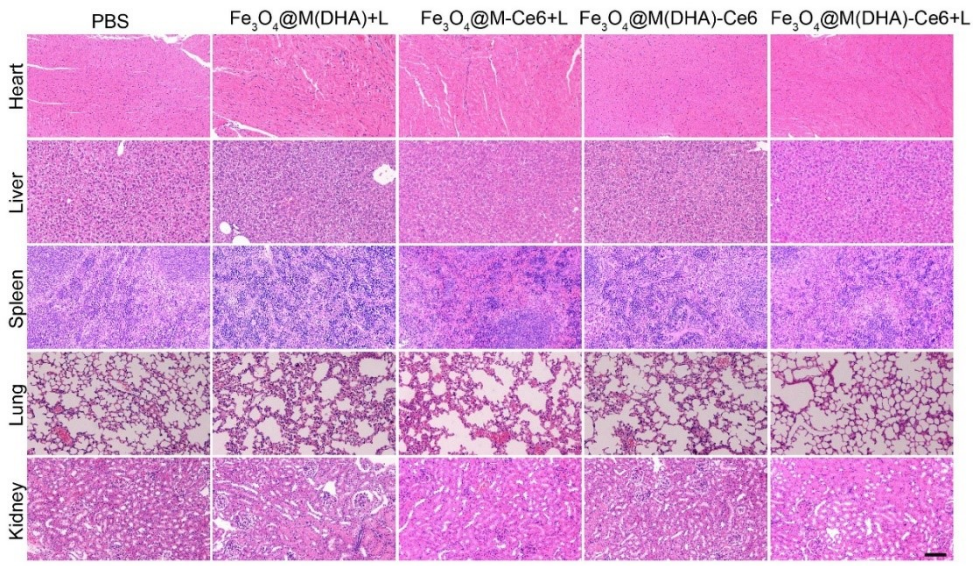

Fig. S13. The HE images of major organs of mice after treatments beginning. The scale bar is 100 μm.

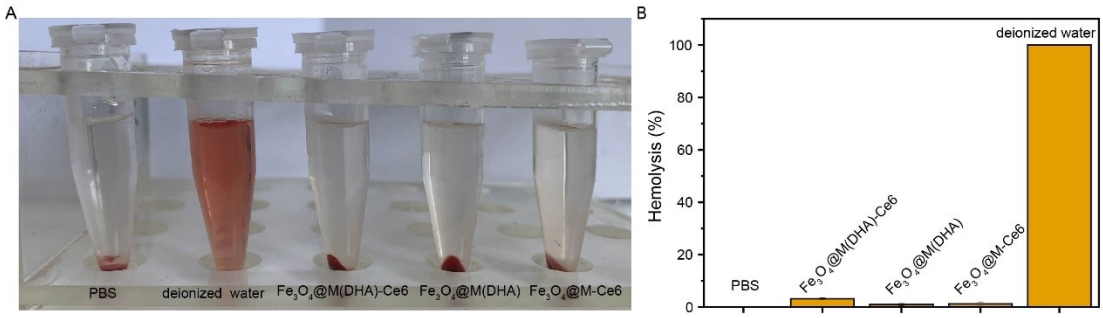

Fig. S14. Hemolysis analysis of Fe_3O_4@M(DHA)-Ce6, Fe_3O_4@M(DHA) and Fe_3O_4@M-Ce6.

References

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