Electronic Supplementary Information

Ce-MOF@Polydopamine Composite Nanozyme Efficiently Scavenges Reactive Oxygen Species and Iron in Thalassemia Disease

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1. Supplementary Methods

1.1 Materials and reagents

No further purification was required for all chemical reagents. Cerium (IV) ammonium nitrate, 1,4-benzendicarboxylic acid (H_2BDC), hydroethidine (HE), sodium chloride (NaCl) and N,N-dimethylformamide (DMF) were purchased from Beijing HWRK Chem Co., Ltd. Hydrogen peroxide (H_2O_2, 30 wt%), penicillin-streptomycin, phosphate buffered saline (PBS), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), fetal bovine serum and DMEM medium and 1-diphenyl-2-picrylhydrazyl (DPPH) were acquired from Shanghai Macklin Biochemical Co., Ltd. Dextran/Fe was purchased Jiangxi Bolai Pharmacy Co., Ltd.

2',7'-dichlorofluorescin diacetate (DCFH-DA) was obtained from Beyotime Institute of Biotechnology. SOD assay kit-WST and mouse ferritin enzyme-linked immunosorbent assay kit were obtained from Sigma-Aldrich. Ethanol (EtOH) and acetone were acquired from Sinopharm Chemical Reagent Co., Ltd. RAW264.7 cells were obtained from Stem Cell Bank, Chinese Academy of Sciences. The female Balb/C mice were acquired from Hunan SJA laboratory animal Co., Ltd. Ultrapure water (Millipore 18.2 MΩ•cm) used throughout the whole experiments.

1.2. Instruments and characterization

Scanning electron microscope (SEM) was performed with a Quanta 200 FEG SEM (Philips, Netherland). Transmission electron microscopy (TEM) photos were obtained by a FEI Tecnai G2 20 with a field emission gun operating at 200 kV. X-ray diffraction (XRD) patterns were characterized on a D/max 2550 VB/PC diffractometer (Rigaku, Japan) using Cu Kα radiation (λ = 0.15418 nm). Fourier transform infrared (FT-IR) was carried out on a spectrometer (PE, USA). X-ray photoelectron spectroscopy (XPS) data were gotten from a Thermo ESCALAB 250XI electron spectrometer. The UV-Vis absorption spectra were recorded on the Cary 60 spectrophotometer (Agilent, USA). The concentrations of Fe were detected by the inductively coupled plasma optical emission spectrometer (ICP-OES) (Flexar/NexlON300X, USA.). The electron paramagnetic resonance (EPR) were detected by JES FA200(JEOL, Japan)).

1.3. SOD mimetic activity assay

The superoxide anion scavenging activity was assessed with a SOD assay kit (Sigma-Aldrich, USA). First, 20 μL different nano concentration was mixed with 160 μL of a 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2*H*-tetrazolium sodium salt (WST-1) working solution. Then, 20 μL of a xanthine oxidase solution was added to each microplate well. After incubating at 37 °C for 20 min, the absorbance at 450 nm was measured using a multiple plate reader (Victor X4). The inhibition rate of the superoxide was calculated by measuring the reduction in color development.

1.4. Catalase-like activity assay.

Fluorescent method H_2O_2 can decompose into ·OH, which would react with terephthalic acid (TA) to produce fluorescent 2-hydroxyterephthalic acid with an excitation wavelength of 320 nm and an emission peak at 425 nm. In the presence of catalase (or catalase mimics), H_2O_2 would decompose into H_2O and O_2 and could not generate fluorescent 2-hydroxyterephthalic acid. Therefore, by monitoring the fluorescent signal of 2-hydroxyterephthalic acid, the elimination of H_2O_2 could be investigated. After 800 μL phosphate buffer (10 mM, pH 7.2) containing H_2O_2 (1 mM) and 100 μL different concentrations of nanoparticle was vortexed vigorously and incubated at room temperature for 5 h, 100 μL TA (0.5 mM) was added. Reaction 1 h, then the fluorescence of the mixture was measured.

1.5. Elimination of iron assay(ICP)

0.1 g Ce-MOF@PDA or 0.1 g Ce-MoF was added to 10 mL PBS solution (pH=7.2) containing 2 mM Fe^{3+}

and incubated at room temperature for 11 h. Incubation at 37 °C for predetermined time, 100 µL of the supernatant was taken out foranalysis and 100 µL of fresh media was added into the well to keep a constant volume. The Fe^{3+} concentration in the supernatant was determined by using inductively coupled plasma (ICP) assay.

1.6. In vitro cytotoxicity studies

The in vitro cytotoxicity of nanoparticles was tested using 3-(4,5-dimethylthiazol- 2-yl)-2,5-diphenyltetrazolium bromide MTT assay. Specifically, RAW264.7 cells were seeded in 96-well plate and were incubated for 24 h. Series dilutions of Ce-MOF@PDA and Ce-MOF ranging from 10 to 100 µg/mL were added into each well and were cultured for another 24 hours. After this, 100 µL MTT solution was added to each well. Incubating with cells for 4 h. MTT was removed, and 100 µL DMSO was added into each well using dissolving the purple formazan crystal. The cell viability was measured through reading the absorbance at 570 nm and samples were normalized to non-treated cells.

1.7. In vitro ferritin reduction assay

RAW264.7 cells were seeded in 6-well plates and and were incubated for 24 h. The cells were treated with culture medium containing 100 µM Ferric ammonium citrate (FAC) for 24 h to induce IO as described above. Subsequently, cells were washed with PBS and treated with Ce-MOF@PDA and Ce-MOF at 10 and 100 µg/mL. After 48 h incubation, cells were lysed with cell lysis buffer (150 mM NaCl, 10 mM Tris, 1% Triton X-100 and protease inhibitor cocktail, pH 7.4). Total protein concentration was measured with the BCA protein assay kit (Sigma-Aldrich, USA) and cellular ferritin concentration was measured with a mouse ferritin ELISA kit (Sigma-Aldrich, USA). The results are plotted as the ratio of ng of ferritin per µg total protein concentration.

1.8. Measuring iron-mediated oxidation stress levels in the presence of iron Ce-MOF@PDA.

The DCFDA cellular ROS detection assay kit was used to determine the capability of Ce-MOF@PDA and Ce-MOF to reduce iron-mediated oxidative stress in iron overload (IO) cells. Briefly, RAW264.7 cells were seeded in 96-well plates at a density of 10,000 cells/well incubated at 37 °C, 5% CO_2 and 100% humidity with DMEM complete medium for 24 h prior to treating with culture medium containing 100 µM ferric ammonium citrate (FAC) for 24 h to induce IO. Subsequently, cells were washed with PBS and treated with 50 µM DFO, 0.1 mg/mL Ce-MOF@PDA) and 0.1 mg/mL Ce-MOF for 8 h; Next, non-fluorescent DCFDA solution was added to each well at a final concentration of 20 µM for 30 min at 37 °C. Cells were washed, and H_2O_2 was added to each well at a final concentration of 50 µM; the fluorescence change was measured at indicated times using a SpectraMax Gemini EM microplate reader by exciting at 485 nm and measuring emission at 535 nm at 37 °C. NIO cells that had not been treated with materials but had been treated with DCFDA served as control blanks.

1.9. In vivo elimination studies

All the animal studies were approved by the Committee for Experimental Animals Welfare and School of Chemistry and Pharmaceutical Science of Guangxi Normal University.. Female Balb/C mice, 6 weeks old, were housed in cages in a room maintained at 20 ± 1 °C and with 12 h light and dark cycles. Feed and water were available ad libitum. Mice were IO by a single tail vein injection of Dextran/Fe (150 mg/kg of Fe, 10 µL/g BW in normal saline) on Day 1. On Day 8, mice were housed into each metabolic chamber and started on iron-deficient powder diet. The following 5 treatments were administered intravenously to animals on Day 8 and every other day for a total of 5 doses: Group 1= NIO control mice received saline; Group 2=IO mice received saline; Group 3=IO mice received 150 mg/kg DFO; Group 4=IO mice received 500 mg/kg Ce-MOF@PDA; Group 5=IO mice received 500 mg/kg Ce-MOF. Mice were monitored daily and bw were recorded on alternate days, and necropsied 7 days following administration of the last treatment dose.

To quantify elimination of iron from animals, feces and urine were collected daily from metabolic chambers and weighed throughout the duration of the study. Fecal material was homogenized in distilled water, iron was extracted from the homogenate by the addition of 5% Trichloroacetic acid and 1.5 M HCl, and the extract was

heated to 70 °C for 90 min. Final iron concentration in urine and feces was measured by standard Inductively coupled plasma mass spectrometry (ICP-MS, Agilent, USA).

Mice we atomic re euthanized by CO_2 over dose and blood was directly collected via cardiac puncture and added to microcentrifuge tubes to separate out the serum for ferritin measurements by ELISA assay based on the manufacturer's instructions. The lungs, heart, spleen, kidneys, brain and liver of animals were subsequently harvested, rinsed with fresh PBS, blotted dry with Whatman filter paper, and then weighed (note that organ weight is reported as mg of total organ weight per g of animal BW, mg/g). Next, organs were sectioned into different portions and snap-frozen in liquid nitrogen or fixed in neutral buffered formalin.

Determination of total iron content in liver: The liver tissue was homogenized, and was digested with mixed acid (nitric acid:hydrogen peroxide:perchloric acid=1:1:3). After the white smoke disappeared, the concentration of iron in the solution was determined by ICP.

Determination of ROS content in liver: The liver tissue was homogenized, centrifuge the supernatant, adding ROS detection probe DCF (the final concentration of DCF is 5 μM) and incubate at 37 °C for 1 hour in the dark, and then use a microplate reader to detect fluorescence strength.

Determination of SOD activity in liver: The liver tissue was homogenized, centrifuge the supernatant, The following steps are consistent with the previous method of detecting SOD mimetic activity simulation.

2. Supplementary Figures and Table

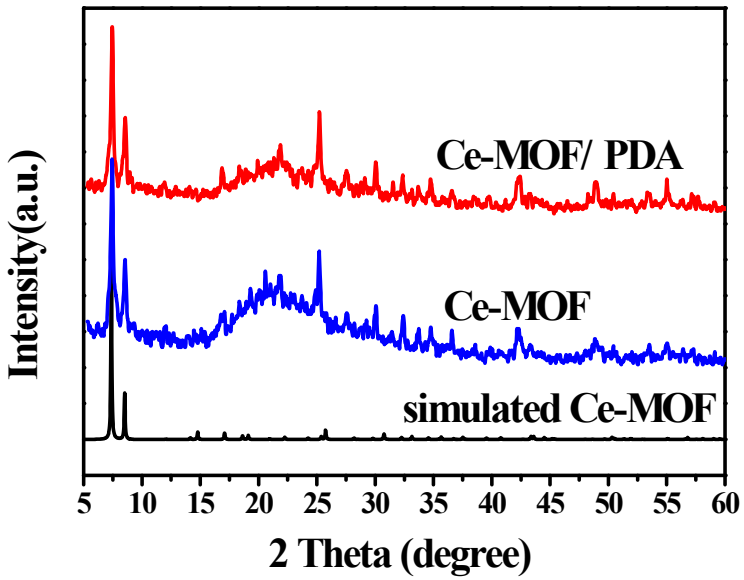


Fig. S1 XRD pattern of Ce-MOF and Ce-MOF@PDA

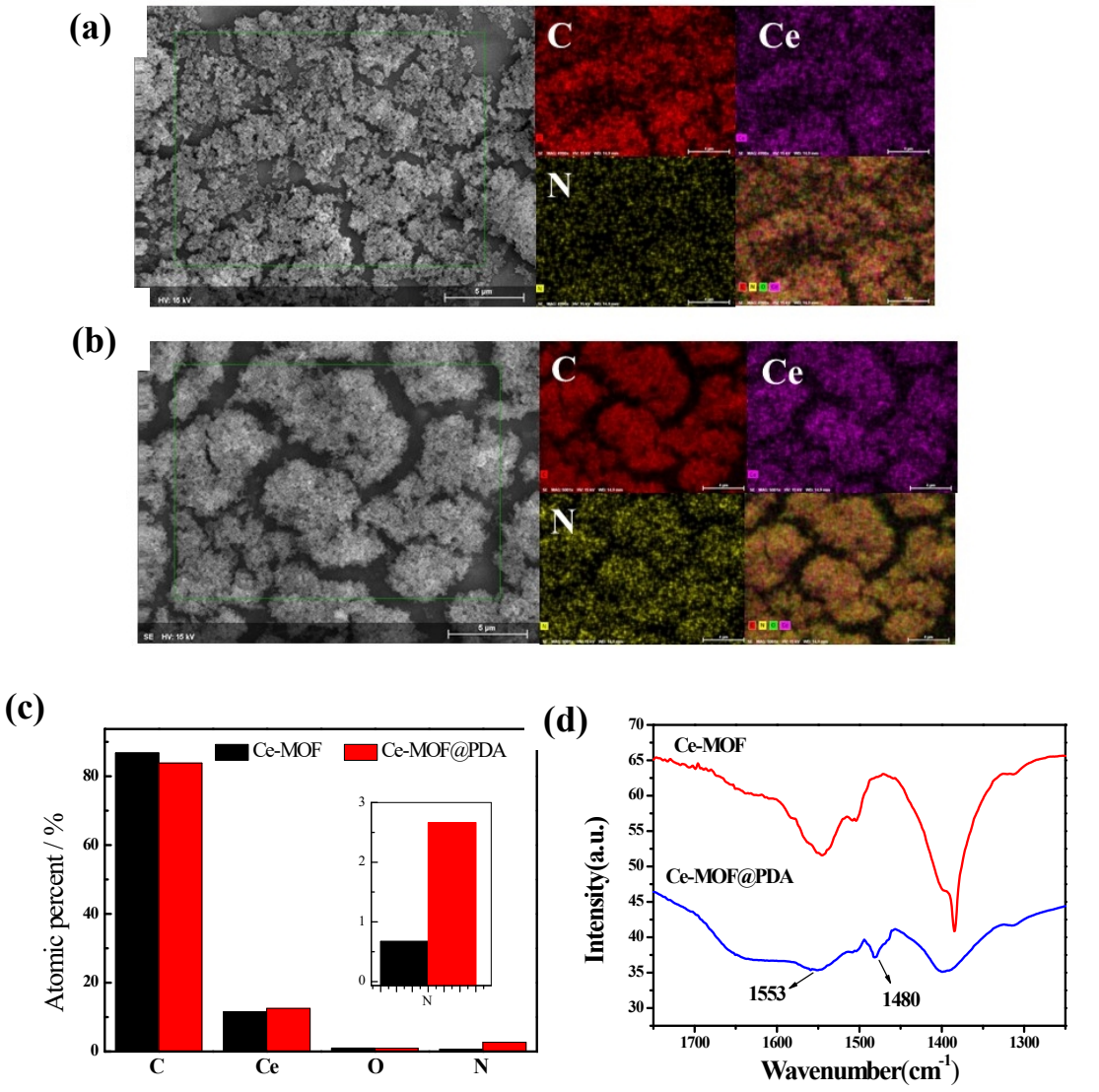


Fig. S2 Energy-dispersive X-ray spectroscopy (EDX) elements mapping of Ce, N and C of Ce-MOF(a) and Ce-MOF@PDA(b); (c) Overlay map showing the distribution of C, Ce, O and N elements in the Ce-MOF and Ce-MOF@PDA; (d) FT-IR spectra of Ce-MOF and Ce-MOF@PDA

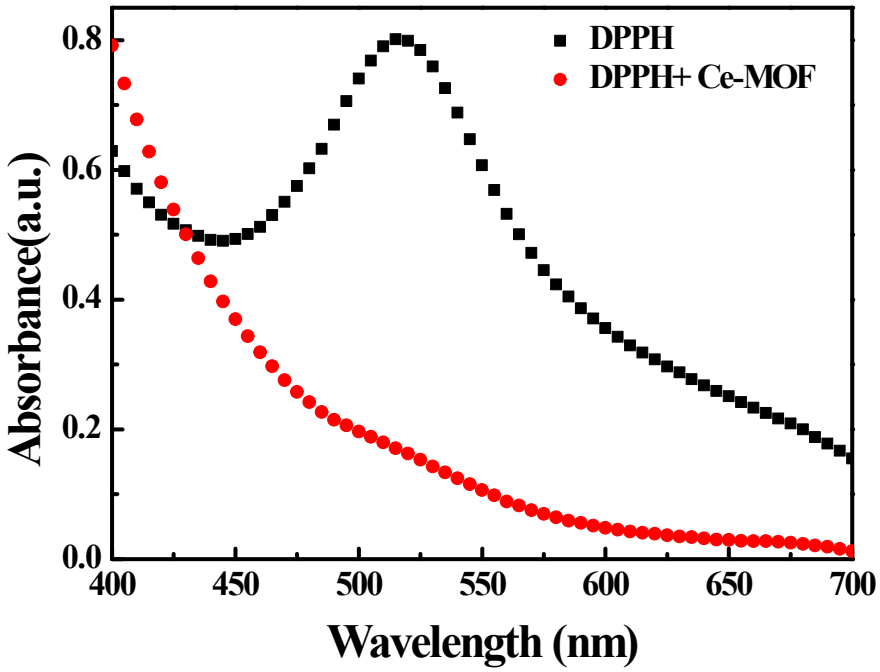


Fig. S3 The UV-Vis spectrogram of ethanolic DPPH solution and discolored after exposure (0.5 h) to the Ce-MOF.

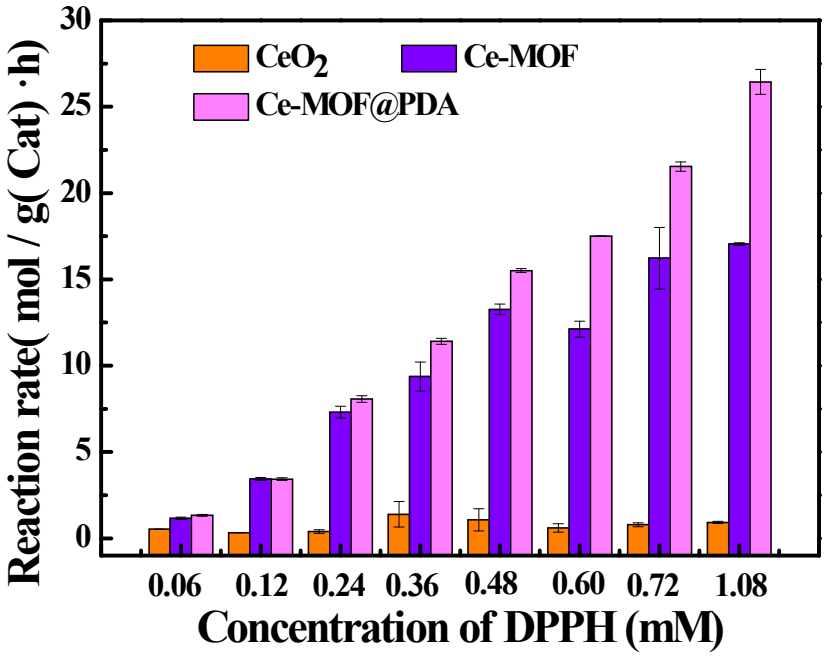


Fig. S4 Reaction rate of the elimination of DPPH and concentrations of DPPH (The material dosage is 50 μg/mL)

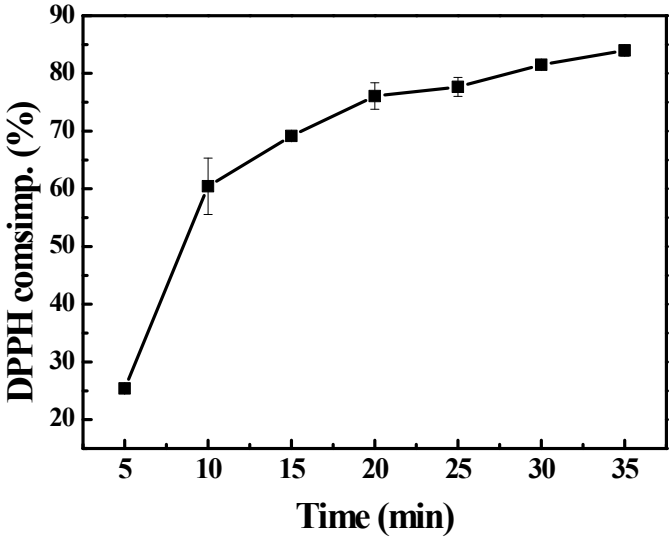
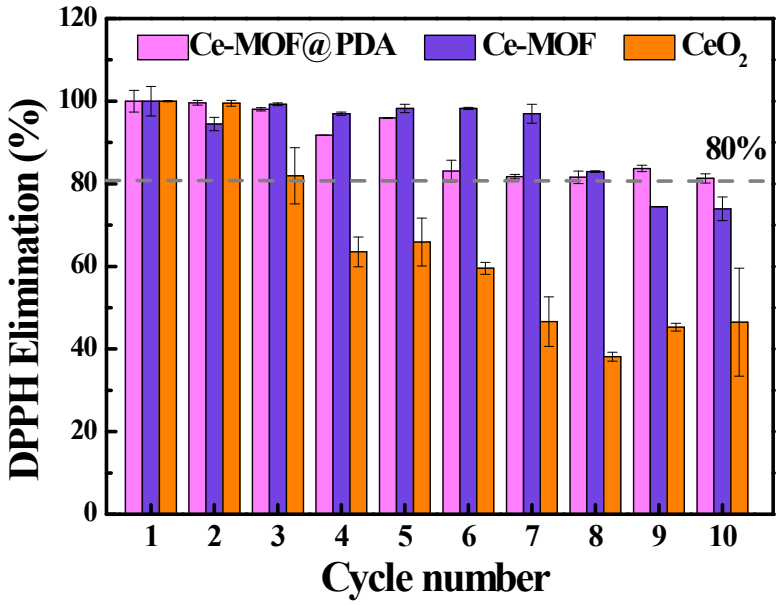


Fig. S5 Effect of time on DPPH elimination rate (concentrations of DPPH is 0.36 mM, the material dosage is 50 μg/mL)



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147 **Fig. S6** Reuse performance Of CeO_2 NPs, Ce-MOF and Ce-MOF@PDA

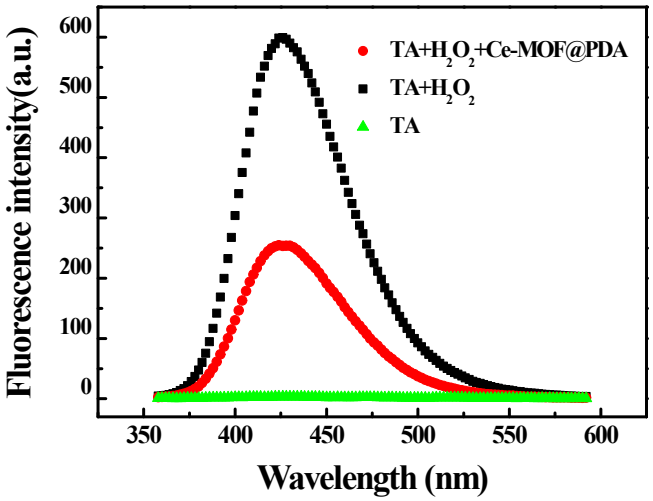

Fig. S7 Fluorescent spectra of TA after reaction with H_2O_2, in the absence and presence of Ce-MOF@PDA.

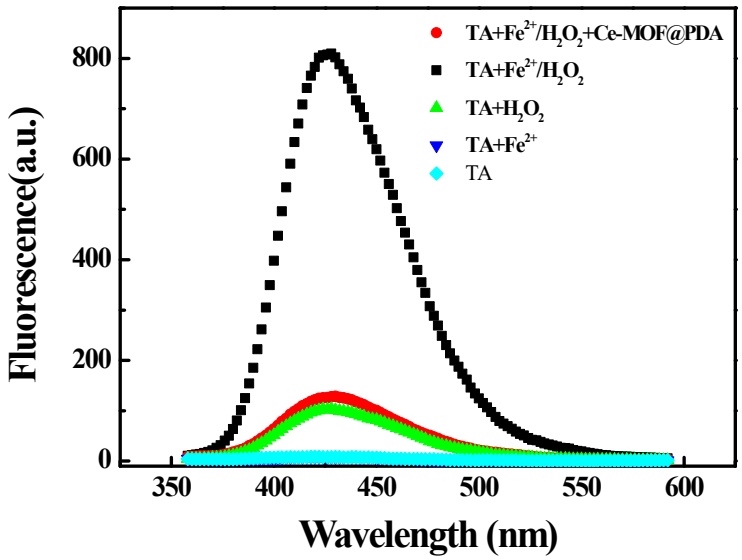

Fig. S8 Fluorescent spectra of TA after reaction with Fe^{2+}/H_2O_2, in the absence and presence of Ce-MOF@PDA. TA alone, and TA reacted with Fe^{2+} or H_2O_2 were used as control.

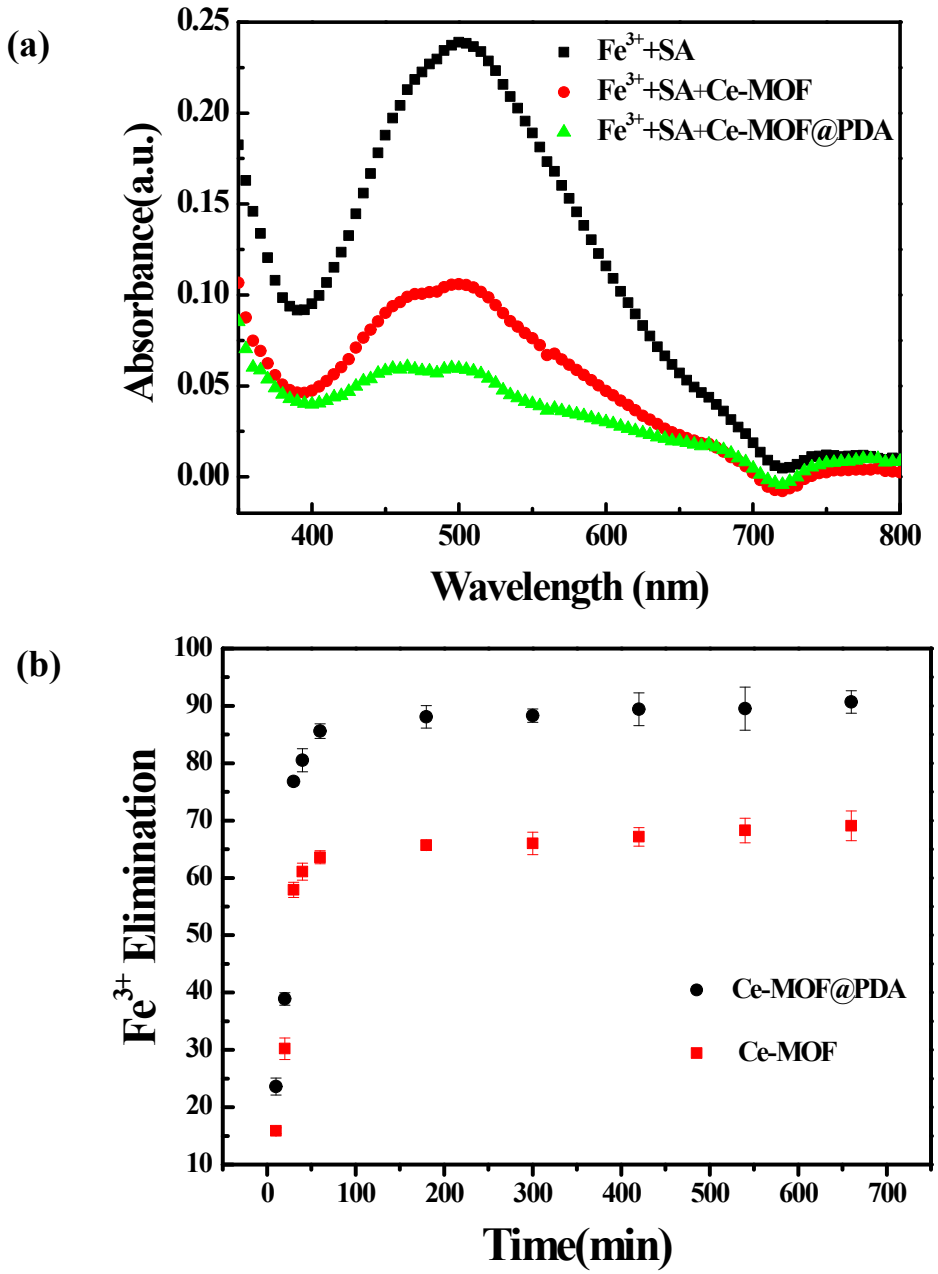

Fig. S9 (a)Absorption spectrum of SA after reaction with Fe^{3+}, in the absence and presence of Ce-MOF@PDA and Ce-MOF; (b) Effect of contact time on the elimination of Fe^{3+} onto Ce-MOF@PDA and Ce-MOF(2 mM Fe^{3+}, 100 μg/mL Ce-MOF@PDA or Ce-MOF).

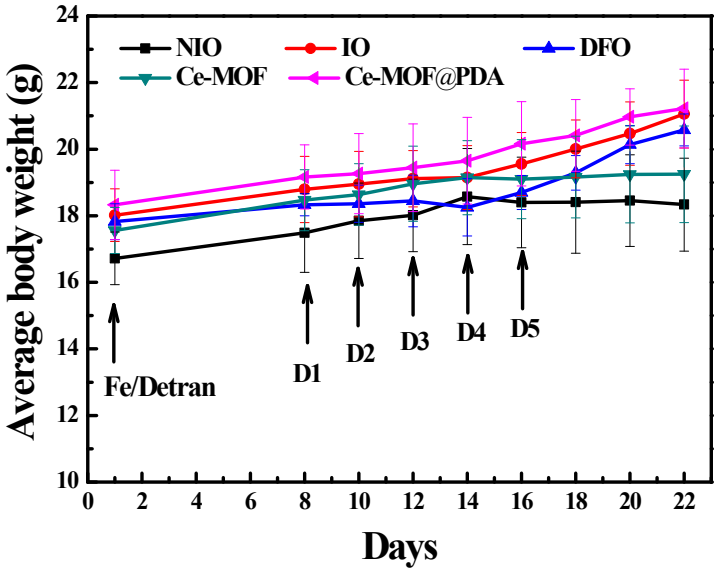

Fig. S10 BW of mice receiving Ce-MOF@PDA, Ce-MOF and DFO treatments

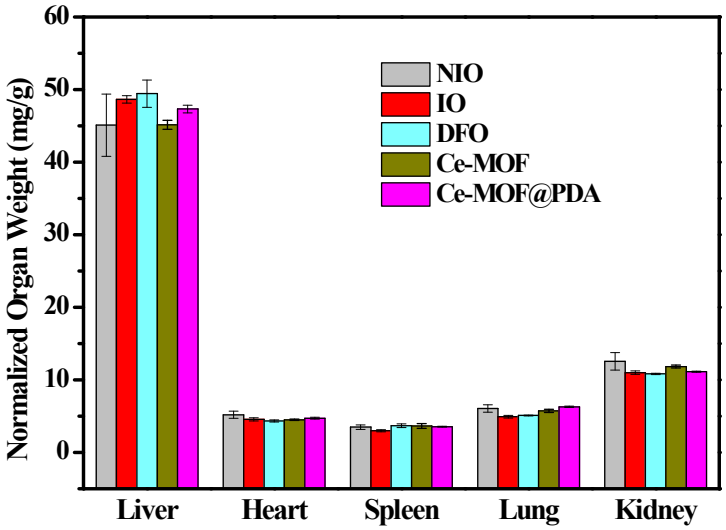

Fig. S11 The normalized organ weights with respect to animal body weight (mg/g)

Table S1 Summary comparing histological lesions in major organs after H&E staining, as assessed by a board certified pathologist.

	Liver	Spleen	Heart	Kidney	Lung
NIO	0	0	0	0	0
IO	++	0	0	0	0
DFO	++	0	0	0	0
Ce-MOF	++	0	0	0	0
Ce-MOF@PDA	+	0	0	0	0

Focal inflammatory focus around hemosiderin-laden cells: +; Multifocal inflammatory sites around hemosiderin-laden cells: ++; Numerous inflammatory foci around hemosiderin-laden cells: +++; Large number of inflammatory foci around hemosiderin-laden cells: ++++.