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Supporting Information

Manganese carbonyl-encapsulated Fe-MOFs as a H_2O_2-responsive CO gas nanogenerator for synergistic gas/chemodynamic therapy

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1. Experimental Section

1.1 Materials

All the chemical reagents and solvents in our research were of analytical grade and used upon receipt without further purification. Sodium dithionite (SDT), 3,3',5,5'-tetramethyl-benzidine (TMB), Glutathione (Reduced) were purchased from Aladdin. 2,2'-bipyridine-4,4'-dicarboxylic acid (Bpydc) was purchased from CHEMBEE. Manganese carbonyl ([MnBr(CO)$_5$], MnCO) was purchased from Alfa Aesar. Hemoglobin (Hb) was purchased from MP Biomedicals. Hydrogen peroxide (H_2O_2) was purchased from XiLong SCIENTIFIC. The methylthiazolyldiphenyl-tetrazolium bromide (MTT) was purchased from BIOFROX. Calcein acetoxymethyl ester (calcein AM), propidium iodide (PI) were purchased from Beyotime. 2,7-dichlorofluorescein diacetate (DCFH-DA) were purchased from FEIYUBIO. $PdCl_2$ were purchased from Energy Chemical. Penicillin-Streptomycin Solution, Dulbecco's modified Eagle's medium (DMEM), TRYPSIN 0.25 % (1X) Solution were purchased from HyClone. Iron(II) acetate、Fetal bovine serum (FBS) was purchased from EVERY GREEN.

1.2 Characterization

Scanning electron microscopy (SEM) images were acquired on a ZEISS Gemini SEM 300. Transmission electron microscopy (TEM) observations were conducted on a FEI Talox F200X electron microscope at an acceleration voltage of 300 kV. The UV-Vis-NIR spectroscopy was recorded by using a Shimadzu UV3600 spectrophotometer. XRD was carried out by means of a Rigaku D/max-2550pc instrument with monochromatized CuKa radiation and a scanning step of 0.028. The zeta potential and dynamic light scattering (DLS) analysis were performed through a Zetasizer Nano-ZS LA-960 instrument (HORIBA, Japan). Confocal laser scanning microscope (CLSM) images were acquired by Leica TCS SP8 confocal laser scanning microscope.

1.3 Synthesis of Fe-MOFs, MnCO@Fe-MOFs, and MnCo@Fe-MOFs@GOx

Synthesis of Fe-MOFs: 121 mg of Bpydc (2,2'-bipyridine-5, 5-dicarboxylic acid) was dissolved in 30 mL of dimethylformamide (DMF), acetic acid (0.572 mL) was dropped, ultrasonically dissolved, then ferric (III) chloride hexahydrate (270 mg) was added, stirred, dissolved, the above solution was transferred to an autoclave, and the

reaction was carried out in an oven at 120 °C for 48 hours. After solvothermal synthesis, natural cooling to room temperature, centrifugation of collected solids, subsequent washing with DMF and methylene chloride. Samples are dried overnight in an oven at 80 °C.

Synthesis of MnCO@Fe-MOFs: The nanocrystal Fe-MOFs (20 mg) was dispersed in anhydrous ethanol (10 mL), and [$MnBr(CO)_5$, MnCO] (30 mg) was added with magnetic stirring. After uniformly mixing at room temperature for 12 h, the mixture was heated to 75°C and kept at this temperature for another 4 h. The Reddish brown product was collected by centrifugation and thoroughly washed with anhydrous methanol to remove the unloading MnCO. Finally, the product was dried under vacuum and kept in the dark.

Synthesis of MnCO@Fe-MOFs@GOx: MnCO@Fe-MOFs@GOx nanospheres were synthesized according to the reported procedure with a little alternation. Briefly, 10 mg MnCO@Fe-MOFs and 5 mg GOx were dissolved in 10 mL deionized (DI) water. Then the mixture was stirred at room temperature for 12 h. After that, reddish brown emulsion was collected by centrifugation, washed with water for several times and dried for used.

1.4 Measurement of the CO release by a hemoglobin (Hb) method

MnCO@Fe-MOFs: The released CO in PBS was detected spectrophotometrically by measuring the conversion of hemoglobin (Hb) to carboxyhemoglobin (HbCO). Firstly, hemoglobin from bovine erythrocytes (4.2 μM final concentration) was dissolved completely in phosphate buffered saline (10 mM, pH 7.4) with different concentrations of H_2O_2. Then, it was reduced by adding fresh SDT (1.6 mg) under a nitrogen atmosphere. An aqueous solution of MnCO@Fe-MOFs (100 μg/mL) was deoxygenated by bubbling with nitrogen gas and then the solution was added into the freshly prepared Hb solution. Immediately, the whole reaction solution (3 mL) was sealed in a 3.5 mL UV quartz cuvette. The UV adsorption spectra of the solution (λ= 350-600 nm) were collected on a Cary 60 UV/Vis spectrophotometer. In order to eliminate influencing factors and to enhance the accuracy, two strong adsorption bands at λ= 410 and 430 nm, which were attributed to HbCO and Hb, respectively, were used

to quantify the conversion of Hb to HbCO. The Beer–Lambert law was used to calculate the concentration of released CO (C_{CO}) according to the following Equation:

$$C_{co} = \frac{528.6 \times I_{410nm} - 304 \times I_{430nm}}{216.5 \times I_{410nm} + 442.4 \times I_{430nm}} C_{Hb}$$

in which C_{CO} and C_{Hb} express the released CO concentration and the initial Hb concentration (4.2 μM), respectively. $I_{410\ nm}$ and $I_{430\ nm}$ express the intensities of the collected spectrum at λ= 410 and 430 nm, respectively.

MnCO@Fe-MOFs@GOx: The measurement of the CO release from the MnCO@Fe-MOFs@GOx is similar to the above-mentioned method. Hemoglobin from bovine erythrocytes (4.2 μM final concentration) was dissolved completely in phosphate buffered saline (10 mM, pH 7.4) with different concentrations of glucose.

1.5 In Vitro Fe^{2+} Release from Fe-MOFs

To measure GSH-triggered release of Fe^{2+} from Fe-MOFs, Fe-MOFs (2.0 mg) were dispersed in phosphate buffer solution (PBS, 2.0 mL) with different concentrations of GSH (0, 0.2, 2 mM). The suspension was dialyzed in buffer medium (pH 7.4, 10 mL) for 24 h (cut-off 12 kDa MW). Aliquots of each 1.0 mL dialysis solution was withdrawn at the selected time intervals and replaced with an equal volume of fresh medium. The released Fe^{2+} in the buffer solution was collected and mixed with phenanthroline solution (50 μL, 100 mM) as Fe^{2+} probe for 15 min. The phenanthroline could react with Fe^{2+} to form complex with absorbance at 512 nm. The content of released Fe^{2+} was measured by UV-vis absorption technique.

1.6 Catalytic activity of MnCO@Fe-MOFs@GOx

Different concentrations of MnCO@Fe-Bpydc@GOx (0, 10, 50 and 100 μg/mL) were added into 1 mg/mL glucose solution, then the oxygen concentrations of glucose solution can be detected by the portable dissolved oxygen instrument. The pH value was recorded with a portable pH instrument.

1.7 Detection of •OH

H_2O_2 could be catalyzed by Fe-MOFs to generate •OH. The •OH radical has strong oxidizability. 3,3'-5,5'-Tetramethylbenzidine (TMB) as an indicator could be oxidized into ox-TMB by •OH, producing an obvious absorption peak at 652 nm. Measurements

were carried out in 3 mL AcOH buffer solution (0.1 M, pH 5.0) containing different concentrations of Fe-MOFs (1, 2, 4, 6 and 8 μg/mL), H_2O_2 (3 mM) and TMB (0.25 mM) at 37 °C for 20 min. This colorless mixture will gradually turn blue. All the samples were tested using a UV-Vis spectrophotometer after the reaction for 1h.

Moreover, •OH as a member of ROS could also be detected by 2′,7′-dichlorodihydrofluorescein diacetate (DCFH-DA). The intracellular •OH generation ability of the Fe-Bpydc, MnCO@Fe-Bpydc and GOx@MnCO@Fe-Bpydc was studied using DCFH-DA which could be oxidized to green fluorescent-dichlorofluorescein by intracellular ROS. Typically, HeLa cells were incubated in the 6-well plate with coverslips for 24 h, and then 1 mL of sample (250 μg/mL) solution was added. After 4 h, DCFH-DA was added and the cells were incubated for further 30 min and then washed with PBS for three times. The fluorescence images were recorded at a wavelength of 488 nm by CLSM.

1.8 In Vitro Cytotoxicity

The cytotoxicity of MnCO@Fe-MOFs@GOx was detected with HeLa cells by a MTT assay. In brief, HeLa cells were seeded into 96-well plates (1×10^4 cells per well) for 24 h, and then incubated with different concentrations of Fe-MOFs, MnCO@Fe-MOFs and MnCO@Fe-MOFs@GOx ranging from 10 to 160 μg/mL for another 48 h. Thereafter, the culture medium was replaced with DMEM (1 mL) and MTT solution (10 μL, 5 mg/mL). After 4 h incubation, the medium was replaced with DMSO (150 μL) and shaken for 10 min to dissolve blue formazan. The absorbance at 450 nm of each well was measured using microplate reader.

1.9 Imaging of CO in Living Cells

HeLa cells were seeded in a 6-well culture plate for one night before cell imaging experiments. In a typical experiment of cell imaging, as controls living cells were incubated with COP-1 probe (5 μM) and a mixture of COP-1 and $PdCl_2$ (5 μM each) at 37 °C for 30 min, respectively, and they were imaged after washing with PBS for three times. For imaging of exogenous CO, HeLa cells were pretreated with PBS, Fe-MOFs, MnCO@Fe-MOFs and MnCO@Fe-MOFs@GOx (10 μM, respectively) for 5 h at 37 °C and then were incubated with a mixture of COP-1 probe and $PdCl_2$ (1 μM each) for

30 min at 37 °C Then, the cells were washed twice with PBS and incubated with Hoechst 33342 (1 mL per well) contained medium for 15 min. After washing the cells with PBS buffer, the cells were imaged.

1.10 Detection of mitochondrial membrane potential

HeLa cells were seeded in 6-well plates (2×10^5 cells per well) and cultured for 24 h. The medium was replaced by fresh medium containing Fe-MOFs, MnCO@Fe-MOFs and MnCO@Fe-MOFs@GOx (50 μg/mL), respectively. After incubation for 6 h, the JC-1 solution (1.0 μg/mL) was added and incubated with cells for 20 min followed by washing with PBS for three times and observation by fluorescence microscopy.

1.11 Live/Dead Staining Experiment

HeLa cells were seeded in 6-well plates (3×10^5 cells per well) and incubated for 24 h. The medium was replaced by fresh medium containing Fe-MOFs, MnCO@Fe-MOFs and MnCO@Fe-MOFs@GOx, and after incubation for 24 h. Cells were washed with PBS and incubated with Calcein AM and PI solution (1 mL per well) contained medium for 30 min. The specific Calcein AM /PI staining working solution is as follows: 1 μL of Calcein AM solution (1000X) and 1 μL of PI (1000X) were added to 10 mL of Assay Buffer (1X) and mixed well. Then, 1 mL of the staining working solution were added to the 6-well plates and incubated at 37 °C for 30 min. Finally, the cells were washed three times with PBS and imaged by CLSM. Green fluorescence of calcein-AM was excited at 488 nm and detected with a 500–550 nm bandpass filter. Red fluorescence of PI was excited at 633 nm and detected with a 660–710 nm bandpass filter.

2. Figures

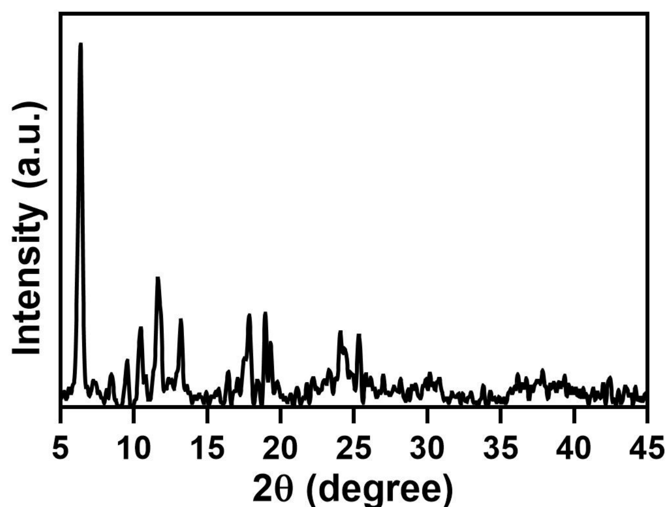

Fig. S1 XRD pattern of Fe-MOFs.

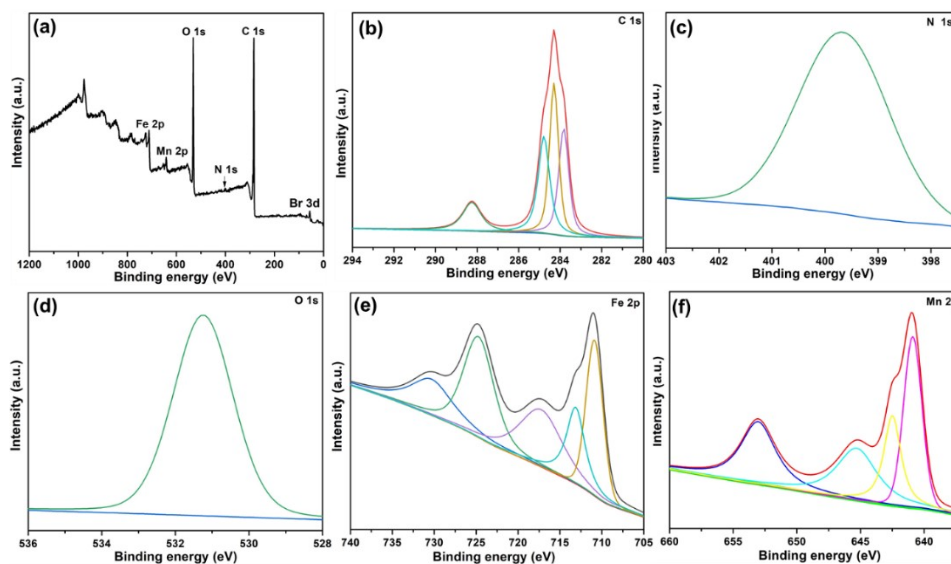

Fig. S2 (a) XPS spectrum of the MnCO@Fe-MOFs composite. High-resolution XPS spectra of (b) C 1s, (c) N 1s, (d) O 1s, (e) Fe 2p, and (f) Mn 2p.

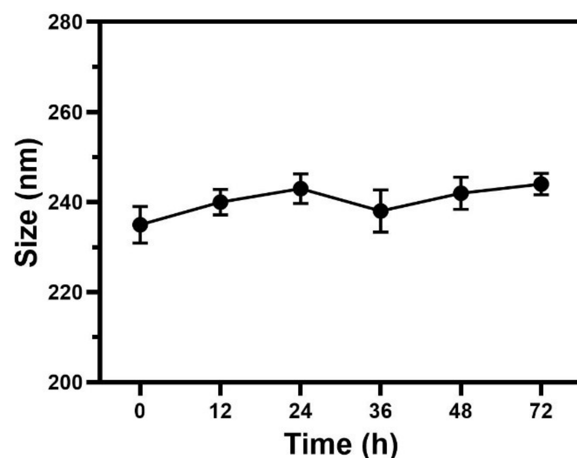

Fig. S3 The mean particle size changes of MnCO@Fe-MOFs@GOx within 3 days in PBS solution (pH=7.4).

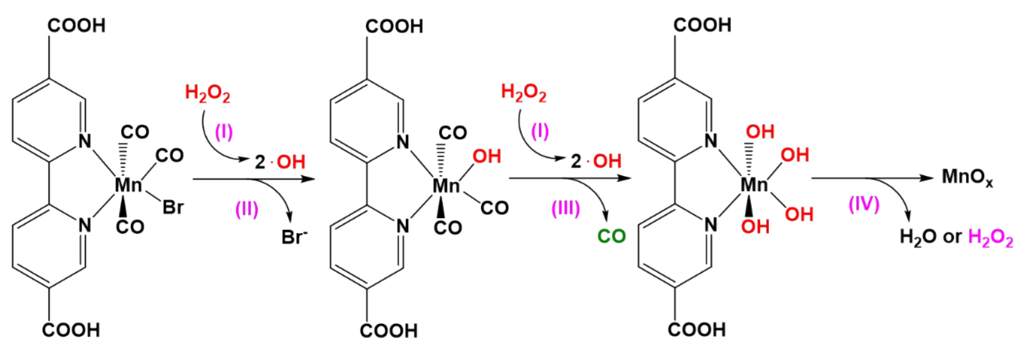

Fig. S4 The proposed mechanism for H_2O_2-responsive CO release from MnCO@Fe-MOFs@GOx involves several key steps: (I) the decomposition of H_2O_2 into ·OH radicals, facilitated by the catalysis of MnCO through a Fenton-like reaction; (II) to (III) the oxidative attacks of ·OH radicals on the most unstable Mn–Br bond and the coordination Mn center, which results in the sequential replacement of carbonyl ligands and the subsequent release of CO; and (IV) the spontaneous decomposition of the generated manganese hydroxyl into MnO_x through the removal of H_2O_2 and H_2O in succession.[1]

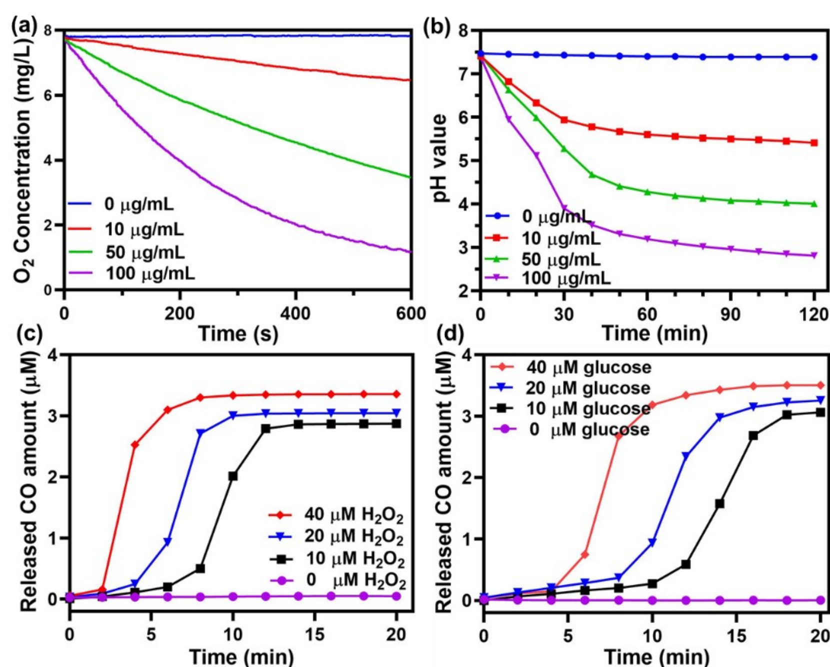

Fig. S5 (a) The oxygen concentration and (b) pH value changes in different concentrations of MnCO@Fe-MOFs@GOx solution with the addition of glucose (1.0 mg/mL). CO gas release profiles of MnCO@Fe-MOFs@GOx in different concentrations of (c) H_2O_2 solutions and (d) glucose solutions.

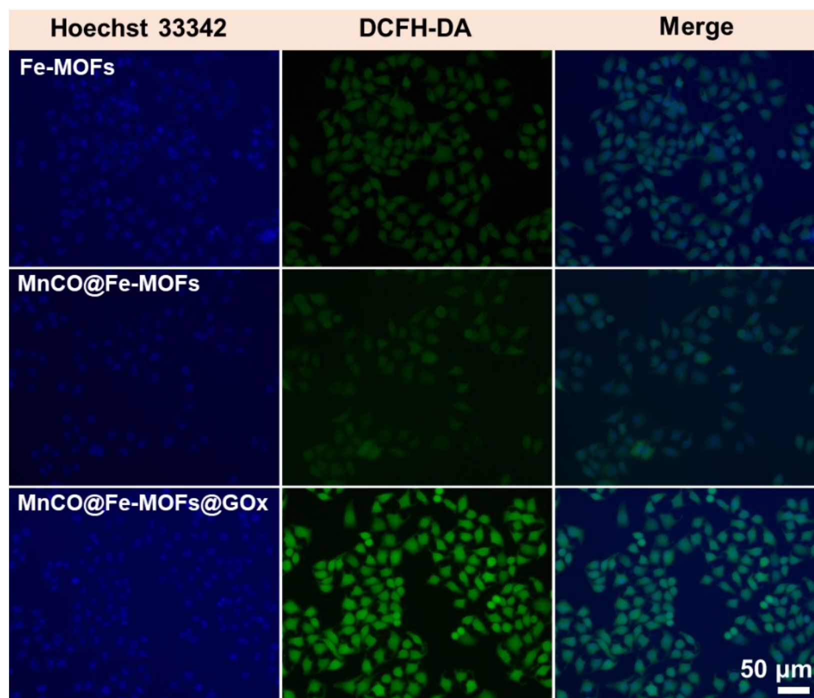

Fig. S6 Fluorescence images of intracellular ROS generation stained HeLa cells treated with Fe-MOFs, MnCO@Fe-MOFs, and MnCO@Fe-MOFs@GOx.

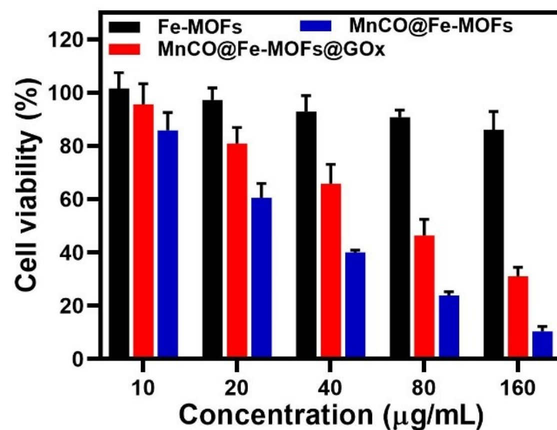

Fig. S7 Cell viability of MCF-7 cells treated with Fe-MOFs, MnCO@Fe-MOFs, and MnCO@Fe-MOFs@GOx.

Reference:

1. Jin, Z.; Zhao, P.; Zhang, J.; Yang, T.; Zhou, G.; Zhang, D.; Wang, T.; He, Q., Intelligent Metal Carbonyl Metal–Organic Framework Nanocomplex for Fluorescent Traceable H_2O_2-Triggered CO Delivery. Chem. Eur. J. 2018, 24, 11667-11674.