Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B

Anti-bacteria and In Vivo Tumor Treatment by Reactive Oxygen Species Generated by Magnetic Nanoparticles

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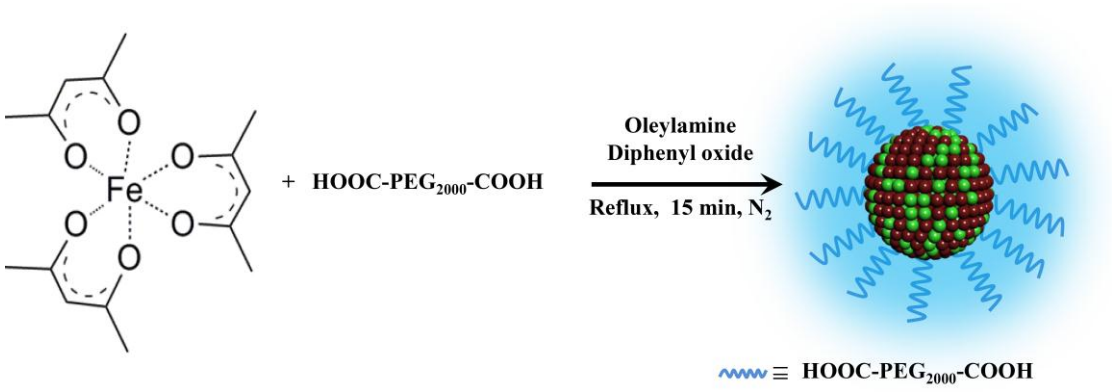
Synthesis of 6-nm MNPs MNPs were prepared using a simple one-pot reaction under a nitrogen atmosphere. Iron (III) acetylacetonate (0.42 g, 6.00 mM), oleyamine (1.58 mL), and HOOC-PEG_{2000}-COOH (4.8 g) were dissolved in diphenyl oxide (20 mL). The solution was heated from room temperature to reflux within 15 min after being purged with nitrogen for 2 h. Under mechanical stirring at 400 rpm, the reaction mixture was refluxed for 15 min. After removing the heat source, the MNPs were precipitated three times with ethanol and ether (v:v = 1:3) until the mixture cool down to room temperature. Finally, the MNPs were dispersed into PBS for further characterizations and applications.

Synthesis of 13-nm MNPs Similar to the synthetic route of preparation of 6-nm MNPs, the 13-nm MNPs was obtained by control of reflux time of reaction mixture in diphenyl oxide for 30 min.



Scheme S1 The method of synthesis of MNPs

Table S1 Hydrodynamic sizes (D_H) of MNPs and their polydispersity index (PDI.) in the PBS buffer (pH = 7.4)

	1 d		60 d	
	D_H (nm)	PDI.	D_H (nm)	PDI.
6-nm MNPs	38.0 ± 12	0.14	38.2 ± 16	0.16
13-nm MNPs	60.0 ± 7	0.20	58.4 ± 9	0.18

Transmission electron microscope (TEM) The morphology and size of 6 and 13-nm MNPs were examined on a Tecnai G2 20 S-TWIN transmission electron microscope at an acceleration voltage of 200 kV. The TEM samples were prepared by drop-coating 2-μL of MNPs solution onto carbon-coated copper grids. The liquid was removed with a filter paper after 1 min.

Dynamic light scattering (DLS) DLS experiments were performed with a Zetasizer Nano instrument (Zetasizer Nano ZS) equipped with a 10-mW helium-neon laser (λ = 632.8 nm) and thermoelectric temperature controller. Measurements were taken at a 90 scattering angle. And the sample was dispersed in the PBS buffers (pH = 7.4).

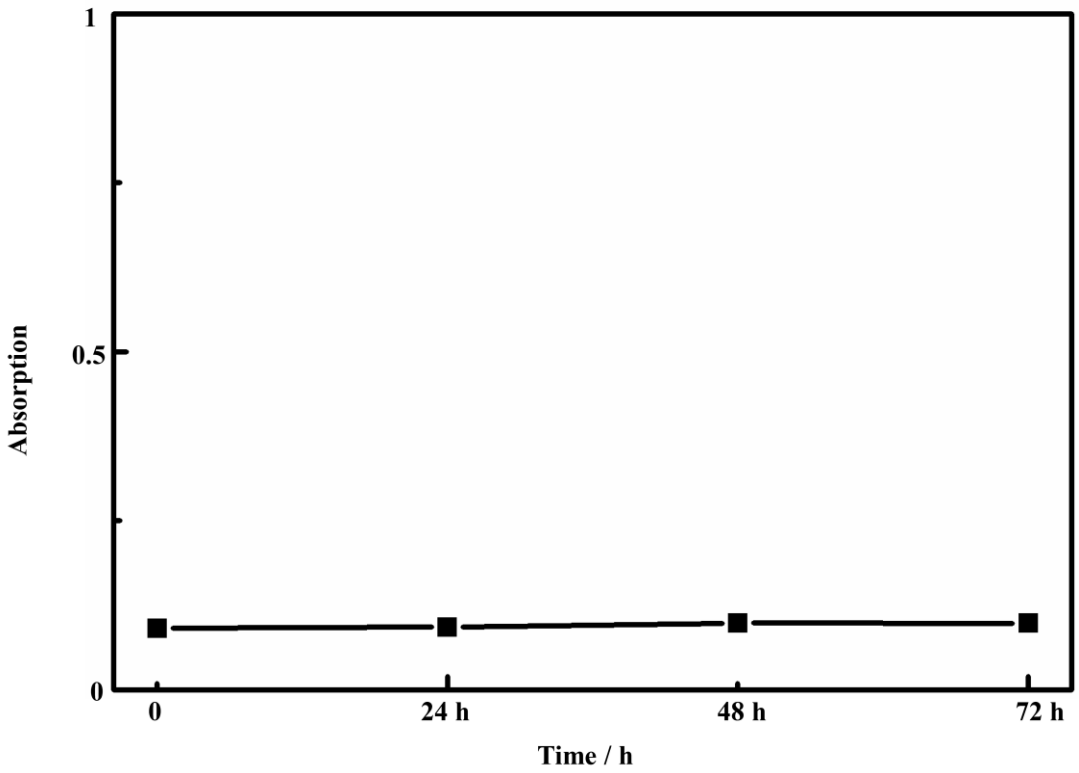


Figure S1 The catalytic activity of disassociated Fe^{2+}/Fe^{3+} in the supernatant was determined by incubation of MNPs in PBS buffer at different time.

Enzyme-mimic activity of 6-nm MNPs in different media To examine the interference of different media on the enzyme-mimic activity of MNPs, we dissolved 6-nm MNPs (100 μg/mL) into LB medium or DMEM. We followed the standard protocol as described above to check the enzymatic activity. As can be seen from Figure S2, the green color of the solutions clearly showed the enzymatic activity of 6-nm MNPs in LB and DMEM.

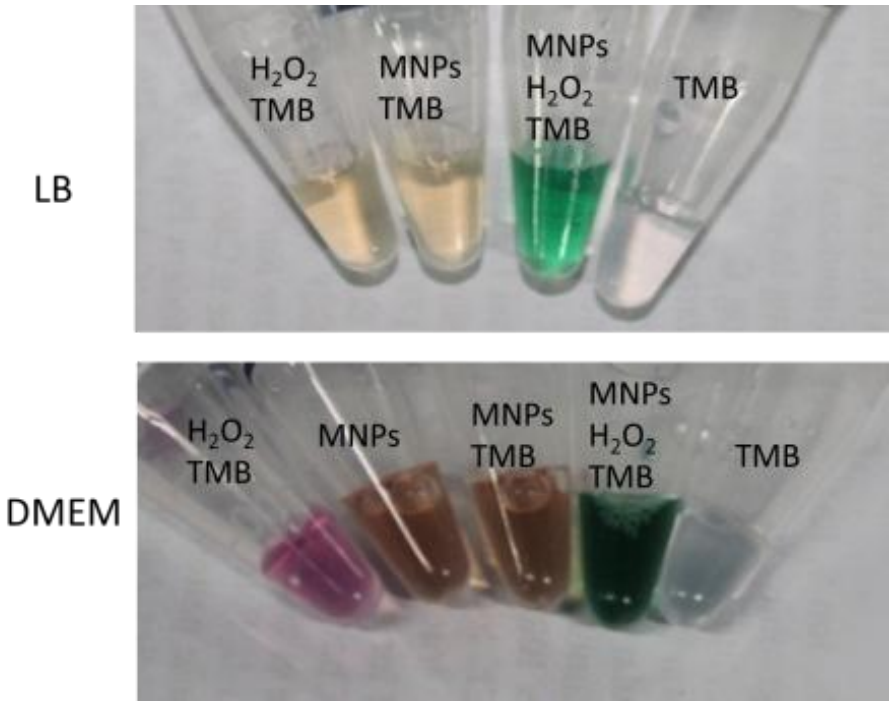


Figure S2 HRP-like activity of MNPs in LB (Luria-Bertani Medium) and DMEM (Dulbecco's Modified Eagle Medium)

Preparation of LB medium Tryptone (10 g), yeast extracts (5 g) and NaCl (10 g) were dissolved with 1000-mL deionized water. The pH value was adjusted to 7.0 with NaOH. And then the mixture was sterilized at 121 ^{o}C for 20 min by autoclaving. The medium was stored at 4 ^{o}C for future applications.

Optimal concentration of 6-nm MNPs and H_2O_2 for bacteria 2-μL cryopreserved *E. coli* was added into LB medium (5 mL) and cultured at 37 ^{o}C for 6 h. The solution of the *E. coli* was diluted with LB medium to constant concentration (1×10^6 CFU/mL) and cultured in 96-well plates with different treatments. The pH values of LB medium were adjusted to 5.5, 6.8 and 7.4 with hydrochloric acid. Firstly, the different concentration of H_2O_2 was investigated at different pH values (pH = 5.5, 6.8 and 7.4). H_2O_2 (8.5 and 13.5 μg/mL) were added into the solution of the *E. coli*, and then $OD_{600\ nm}$ of the *E.coli* solution was monitored using microplate reader. At the same time, we evaluated the cytotoxicity of different concentration of 6-nm MNPs. In the experiment of investigate the anti-*E. coli* activity dependent on the concentration

of MNPs, different concentration of 6-nm MNPs (8 and 20 μg/mL) were added into the solution of *E. coli* when the concentration of H_2O_2 was 13.5 μg/mL at different pH values (pH = 5.5, 6.8 and 7.4).

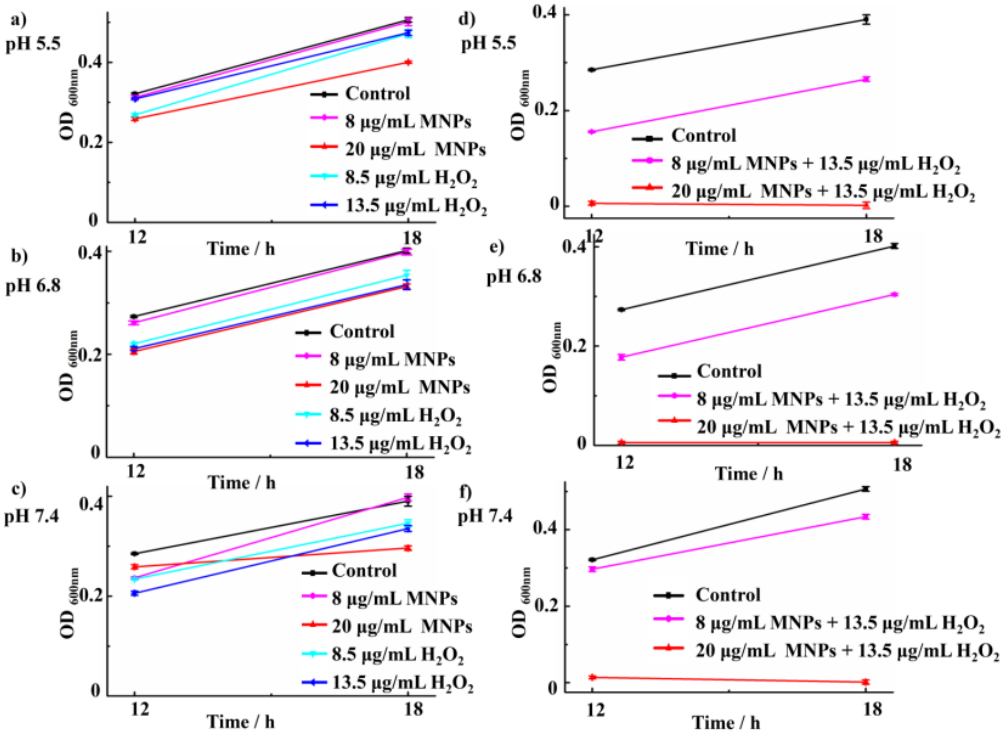


Figure S3 Anti-bacterial activity of 6-nm MNPs in presence of H_2O_2. The cytotoxicity of different concentration of MNPs or H_2O_2 alone at pH = 5.5 (a), 6.8 (b) and 7.4 (c). At the concentration of H_2O_2 (13.5 μg/mL), the anti-bacterial activity was dependent on the concentration of 6-nm MNPs (8 and 20 μg/mL) at pH = 5.5 (d), 6.8 (e) and 7.4 (f).

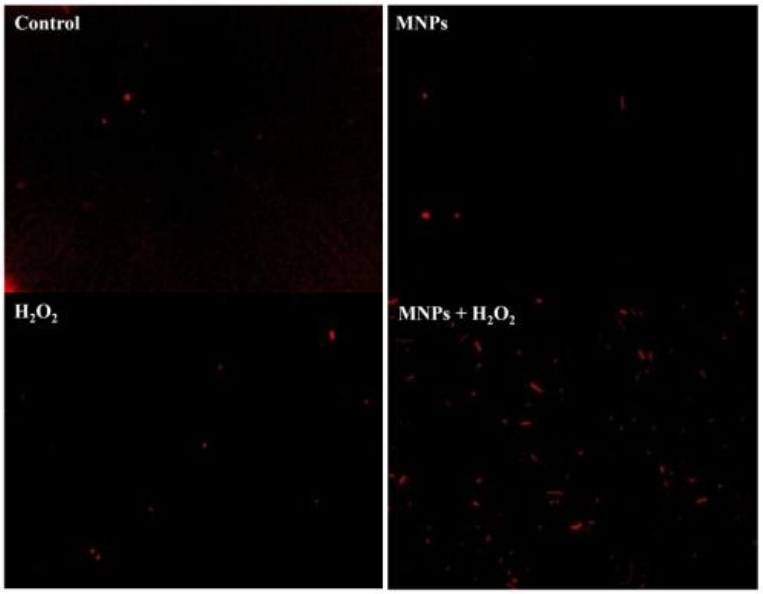


Figure S4 Monitoring dead of *E. coli via* staining of propidium iodide (PI).

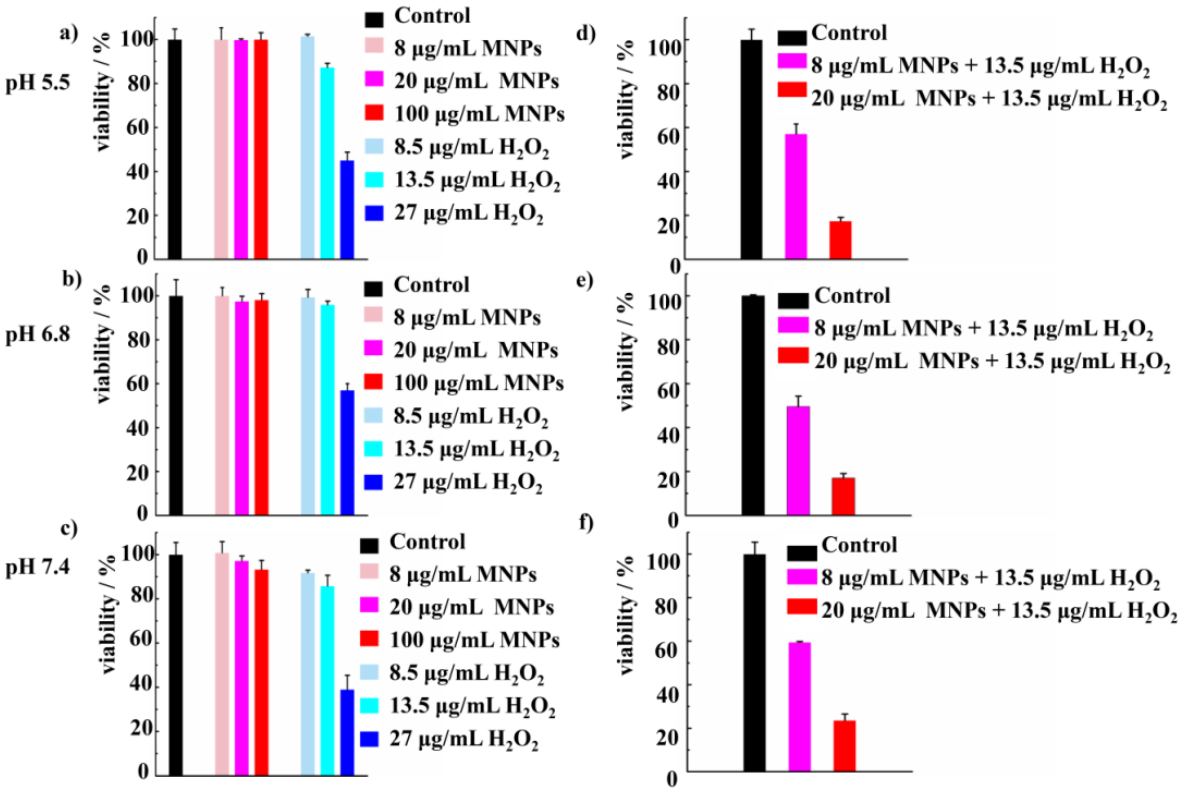


Figure S5 Anti-HeLa cell of MNPs in presence of H_2O_2. The cytotoxicity of different concentration of MNPs and H_2O_2 at pH = 5.5 (a), 6.8 (b) and 7.4 (c). At the concentration of H_2O_2 (13.5 μg/mL), the anti-cancer cell was dependent on the concentration of MNPs (8 and 20 μg/mL) at pH = 5.5 (d), 6.8 (e) and 7.4 (f).

Fluorescence microscope of the *E. Coli* The further investigated the anti-bacterial activity of 6-nm MNPs in the presence of H_2O_2. The dead *E. coli* was investigated with fluorescence microscope (Leica DMI 6000B). During the experiment, *E. coli* was incubated to 6×10^8 CFU/mL, and then MNPs (20 μg/mL) and H_2O_2 (13.5 μg/mL) was added into the medium for killing *E. coli*. The solution was centrifuged at 3000 rpm for 5 min after 1 h treatments, the supernatant was removed and *E. coli* was dispersed into the solution of PI (10 μg/mL) for 10 min. And then fluorescence microscope was employed to investigate the membranous permeability of *E. coli* under different treatments.

The optimal concentration of MNPs and H_2O_2 on anti-cancer cells HeLa cells were cultured in DMEM supplemented with 10% FBS at 37 °C in a humified atmosphere containing 5% CO_2. And then the cells were seeded in 96-well plates at a density of 1×10^5 cells per well at different pH values (pH = 5.5, 6.8 and 7.4). After 15 h adhesion, the 6-nm MNPs was added into the medium and cultured for 1 h. And then H_2O_2 was added into the medium and cultured for another 2 h. Finally, the cell viability of different treatments was investigated by the cell counting kit-8 assay (CCK-8). To investigated the cytotoxicity of the MNPs or H_2O_2 alone added into the medium of cells. Different concentration of 6-nm MNPs (8, 20 and 100 μg/mL) or H_2O_2 (8.5, 13.5 and 27 μg/mL) were added into the wells at different pH values (pH = 5.5, 6.8 and 7.4), the cell viability was investigated after 2 h incubation. At different pH values, however, the anticancer cell activity was dependent on the concentration of MNPs when the concentration of H_2O_2 was 13.5 μg/mL. In these experiments, MNPs (8 and 20 μg/mL) was added into the medium cultured with cell at different pH. The cell was then cultured 1 h at 37 °C in a humified atmosphere containing 5% CO_2. H_2O_2 (13.5 μg/mL) was added into the medium and cultured 2 h. And then the cell viability was examined by CCK-8 assay.

CCK-8 assay Cell viability was investigated by the CCK-8 (cell counting kit-8) assay. The culture medium containing cells and MNPs was incubated for 2 h after addition of H_2O_2 (13.5 μg/mL), the supernatant was removed and washed with PBS twice, and then 200-μL fresh culture medium supplemented with 10% FBS (fetal bovine serum) and 1% penicillin and streptomycin were added into the wells. Subsequently, the solution of CCK-8 (20 μL) was added to the wells followed by incubation for 4 h at 37 ^{o}C in a humified atmosphere containing 5% CO_2. Finally, the absorbance values of the cells per well were determined with a Microplate reader at 419 nm for analyzing the cell viability. Control experiments were done with addition the same volume and concentration of PBS, MNPs or H_2O_2 alone, and the other treatments were under the same condition.

The cell viability was calculated through the equation as follow:

The cell viability = As / Ac × 100%

Where the As was the absorbance of the different treatments with MNPs and H_2O_2, MNPs or H_2O_2 alone. Ac was the absorbance of the control treatment with PBS.

ICP-AES analysis Determination of 6-nm MNPs content in organs was performed by ICP-AES analysis (ELAN 6100, Perkin-Elmer SCIEX). Organs were dissolved into aqua regia. The solution of the resulting was diluted with 2% HNO_3 to constant volumes.

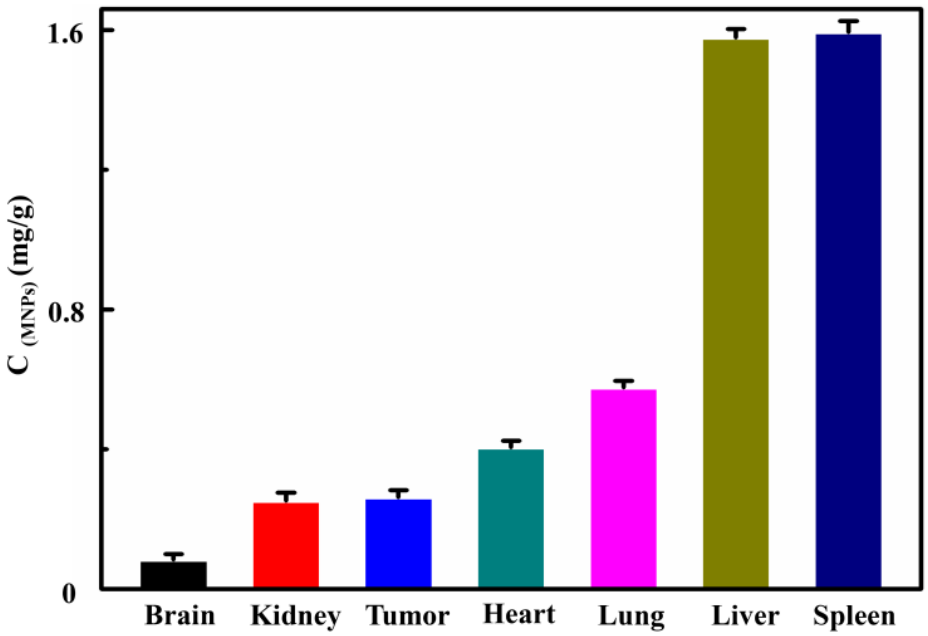


Figure S6 Biodistribution of 6-nm MNPs *in vivo*. Values are expressed with the means ± SD.