Supporting Information

FePt@MnO-Based Nanotheranostic Platform with
Acidity-Triggered Dual-Ions Release for Enhanced MR
Imaging-Guided Ferroptosis Chemodynamic Therapy

Baochan Yang,†,‡,# Qingyun Liu,‡,# Xiuixiu Yao,‡,# Dongsheng Zhang,† Zhichao Dai,†
Ping Cui,† Gaorui Zhang,§ Xiuwen Zheng,*† Dexin Yu*,§

†Key Laboratory of Functional Nanomaterials and Technology in Universities of
Shandong, College of Chemistry and Chemical Engineering, Linyi University, Linyi,
276000, P. R. China.

‡College of Chemical and Environmental Engineering, Shandong University of Science
and Technology, Qingdao, 266590, P. R. China.

§Radiology Departments, Qilu Hospital of Shandong University, Jinan, 250000, P. R. China.

#These authors contribute equally to this work.

*Corresponding authors:

E-mail: zhengxiuwen@lyu.edu.cn.

E-mail: ydx0330@sina.com.

S-1
Methods

Synthesis of manganese oleate. 40 mmol MnCl$_2$·4H$_2$O and 80 mmol sodium oleate were dissolved in 200 mL methanol. Then, 200 mL methanol including 80 mmol NaOH was added dropwise to above solution to precipitate manganese oleate. Finally, the dark brown powder was obtained after product was washed with water, ethanol, and acetone and subsequently dried in high vacuum at 120 °C for 48 h.

The exploration of suitable quality ratio of DSPE-PEG5000-FA coating on FePt@MnO NPs. UV−vis spectroscopy was obtained of DSPE-PEG5000-FA in water at different concentration of 0.5, 0.57, 0.67, 0.8, 1.0 and 2.0 mg/mL (Figure S3A). Then, the standard coordinate line was got based on absorbance intensity at 280 nm of folic acid of Figure S3A (Figure S3B). After coated DSPE-PEG5000-FA on FePt@MnO NPs at quality ratio of 4:1, 6:1, 8:1, 10:1 and 12:1, the UV−vis spectroscopy of supernatant was detected. The results showed the peak intensity of supernatant was small at low quality ratio (4:1, 6:1, 8:1 and 10:1) and smaller growth difference was within the normal range, which indicated nearly all of DSPE-PEG5000-FA was coating on FePt@MnO NPs. When quality ratio was increased to 12:1, the peak intensity of supernatant was suddenly risen, which revealed saturated coating and residuum of DSPE-PEG5000-FA in supernatant (Figure S3C and S3D). In addition, the TEM images and solution images shown in Figure S3E and Figure S3F, which showed (FePt@MnO)@DSPE-PEG5000-FA were monodispersed and no precipitate at quality
ratio of 12:1. These results indicated suitable quality ratio was 12:1 of DSPE-PEG5000-FA coating to FePt@MnO NPs.

**Prussian Blue Staining.** HeLa cells were incubated with FMDF NPs for 15 h at IC₅₀ of Fe. Then, cells were washed three times with PBS and fixed with 4% paraformaldehyde solution for 20 min. After that, Pearls agent (4% potassium ferrocyanide and 12% HCl, 50:50 v/v) was added for 30 min and 1% neutral red for 30 min. The cells were washed well with PBS and examined with an inverted microscope (Olympus IX 53). HeLa cells incubated with the FMDm NPs were carried out with same method as above.
Figure S1. Size distribution of (A) FePt NCs and (B) FePt@MnO NPs measured by DLS in hexane.

Figures
Figure S2. (A) The magnetic properties of FePt NCs and FePt@MnO NPs at room temperature (298K). (B) EDS spectra of FePt@MnO NPs.

Figure S3. The exploration of suitable quality ratio of DSPE-PEG5000-FA coating on FePt@MnO NPs. (A) UV–vis absorption spectra of DSPE-PEG5000-FA in water at different concentration. (B) The standard coordinate line of intensity at 280 nm of FA of (A). (C) After coated DSPE-PEG5000-FA on FePt@MnO NPs at quality ratio of 4:1, 6:1, 8:1, 10:1 and 12:1, the intensity of supernatant at 280 nm of folic acid. (D) The value of peak intensity at 280 nm of (C). (E) TEM images of FMDF NPs at different quality ratio.
of 4:1, 6:1, 8:1, 10:1 and 12:1. (F) The stability image dispersed in PBS of FMDF NPs at different quality ratio after 24 h.

Figure S4. UV-Vis absorption spectra of FMDm NPs.

Figure S5. (A) The visible images of FePt@MnO NPs before and after transferred from lipophilic to hydrophilic by coating DSPE-PEG5000-FA and DSPE-mPEG5000 via solvent-exchange method (the upper layer is hexane, the lower is the water). (B) The stability images dispersed in various biological intermediate solutions of FMDF NPs and FMDm NPs.
FMDm NPs. (C) The TEM images before and after negative staining with 1% PTA (phosphotungstic acid) at pH=7.0 of FMDF NPs and FMDm NPs.

Figure S6. Size distribution of (A) FMDF NPs and (B) FMDm NPs measured by DLS in water.
Figure S7. The fluorescence spectra of DCF after cultured 5µM DCFH-DA, different concentration of FMDF NPs with 10 mM H$_2$O$_2$ at 2 h at pH=5.8.

Figure S8. Fluorescent images of DCFH-DA (for ROS) and Hoechst 33342 (for cell nucleus) of 4T1 cells, HepG2 cells and MCF-7 cells after treated with FMDF NPs at half-maximum inhibitory concentration (IC$_{50}$, [Fe]=40 µg·mL$^{-1}$) for 8 h (scale bars: 200 µm).
Figure S9. (A) The fluorescence spectra of DCF after FMDF NPs at IC_{50} cultured with HeLa cells at various incubation times (0, 3, 6, 9, 12, 15, 18 and 24h). (B) The value change of intensity at 525 nm of DCF of (A).

Figure S10. T1/T2-weighted MR imaging of HeLa cells after cultured with FMDF NPs at IC_{50} at different time (3, 6, 9, 12, 15 and 18 h).
Figure S11. Prussian blue staining assay and neutral red staining (stain cytoplasm of viable cells) were conducted to detect Fe\(^{3+}\) after HeLa cells were treated with FMDF NPs and FMDm NPs for 15 h at IC\(_{50}\) ([Fe]=40 μg·mL\(^{-1}\)) (scale bars: 50 μm).

Figure S12. (A) CT imaging at before and 4 h of 4T1 tumor-bearing balb/c mouse after intratumorally injected with FMDF NPs. (B) Corresponding change of CT imaging signal value (HU) of (A).
Figure S13. In vivo antitumor study with intratumor injection. (A) Measure method of tumor size with a vernier caliper. (B) Eventual tumor mass and photographs (insert) of tumors for four various treatments. (C) Statistical weights of balb/c mice of four different treatments. (D) The biodistribution of Fe and Mn in the major organs of four various treatments measured by ICP-MS. (E) The photographs of major organs after various treatments. (F) The H&E staining images of major organs of four various treatments (scale bars: 200 μm).
Figure S14. In vivo ROS generating induced by FMDF NPs was investigated with tumor-bearing balb/c mice and ROS fluorescent probe DCFH-DA using an intravital real-time confocal microscope system (IVRTCLSM).

Figure S15. In vivo antitumor study with intravenous injection. (A) The compared photographs of tumors before and after various treatments (scale bars: 1 cm). (B) Statistical weights of balb/c mice of different treatments. (C) The H&E staining images of major organs of various treatments (scale bars: 100 μm).
Tables

Table S1. Mass ratio and atomic ratio of FMDF NPs by ICP-MS.

<table>
<thead>
<tr>
<th></th>
<th>Mass ratio</th>
<th>Atomic ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fe</td>
<td>0.374</td>
<td>0.614</td>
</tr>
<tr>
<td>Pt</td>
<td>0.500</td>
<td>0.258</td>
</tr>
<tr>
<td>Mn</td>
<td>0.076</td>
<td>0.127</td>
</tr>
</tbody>
</table>