Supporting Information

Tumor-penetrating iron oxide nanoclusters for \( T_1/T_2 \) dual mode MR imaging-guided combination therapy

Jing Li\(^1,a\), Siman Gong\(^1,a\), Shiyu Li\(^a\), Xinchong Li\(^a\), Siyi Lan\(^a\), Minjie Sun\(^*,a\)

\(^a\)State Key Laboratory of Natural Medicines, Department of Pharmaceutics, China Pharmaceutical University, Nanjing 210009, China.

*Corresponding author

E-mail address: msun@cpu.edu.cn (M. Sun)

\(^1\)The first two authors contributed equally to this paper.
Figure S1. The 1H-NMR spectra of HA (A) and HA-DA (B).
Figure S2. (A) Frequency curve of size distribution of Fe$_2$O$_3$ nanoparticles. Inset: TEM image of Fe$_2$O$_3$ nanoparticles. (B) Frequency curve of size distribution of Fe$_2$O$_3$ nanoparticles and Fe$_2$O$_3$@PDA nanoparticles. (C) TEM image of Fe$_2$O$_3$@PDA.
Figure S3. FESEM image and corresponding elemental composition images of Fe$_3$O$_5$@PFH NCs.
Figure S4. Change in particle size of Fe$_2$O$_3$@PFH nanocluster after incubating in PBS buffer solution (pH 7.4) and 10% plasma.
Figure S5. (A) UV-vis spectra of the Fe$_2$O$_3$@PFH nanocluster. (B) The temperature change of the Fe$_2$O$_3$@PFH NCs solution (0.50 mg/mL) upon four circles of NIR laser irradiation (808 nm, 1.6 W/cm$^2$) that was repeated on and off at a cycle of 750 s. (C) The temperature change of the Fe$_2$O$_3$@PFH nanocluster under laser irradiation (808 nm, 1.6 W/cm$^2$). The laser was switched off until the temperature reached the peak. (D) Linear time data from the cooling period (after 500 s in Figure S5C) vs. negative natural logarithm of driving force temperature. The slope of the linear line was the time constant for heat transfer of the system (364.72 s).
Figure S6. (A) Fluorescence measurement of mixture of Fe$_2$O$_3$@PFH and DOX with different weight ratio at 488 nm. (B) Encapsulation efficiency and drug loading content of Fe$_2$O$_3$@PFDH with the different ratio (w/w). (C) Particle size distribution and (D) zeta potential of Fe$_2$O$_3$@PFDH detected by DLS.
Figure S7. The release curve of DOX from Fe$_2$O$_3$@PFDH NCs under the different conditions of pH 7.4, pH 6.8, PBS or pH 6.8 PBS with HAase (1 mg/mL) at 37 °C.
Figure S8. Prussian blue staining images of the tumor section from mice upon 24 h of injection of saline.
**Figure S9.** Cell viability of H9C2 and Podo cells after incubation with different concentrations of Fe$_2$O$_3$@PFH(A) and HA-DA(B).
Figure S10. Cell viability of 4T1 cells treated by free DOX and Fe$_2$O$_3$@PFDH after incubation for 24h. (n=3)
Figure S11. Fluorescence images of calcein AM (live cells, green) and PI (dead cells, red) co-stained 4T1 cells after treatments with 0.2 mg/mL of Fe$_2$O$_3$@PFH NCs with or without laser irradiation.
**Figure S12.** The fluorescence intensity of heart, liver, spleen, lung, kidneys and tumor isolated at 24 h post-injection of the Cy7-Fe$_2$O$_3$@PFH NCs.
Figure S13. The distribution of the free Cy7-NHS ester with tween 80 in vivo. (A) Images of mice following intravenous injection of the free Cy7. (B) Images of heart, liver, spleen, lung, kidneys and tumor isolated at 24 h post-injection of the free Cy7. (C) The corresponding fluorescence intensity.
Figure S14. Content analysis of DOX in major organs and tumor isolated at predetermined time post-injection of the free DOX.
**Figure S15.** The reciprocal values of relaxation times ($R_1$, $R_2$) of $\text{Fe}_2\text{O}_3$ nanoparticle versus different concentrations of $\text{Fe}^{3+}$, and the relaxivity values ($r_1$, $r_2$) were obtained from the slopes of linear fits of experimental data.
Figure S16. Body weight change of 4T1 tumor-bearing mice treated with different preparations.
**Figure S17.** H&E staining images of major organs from healthy mice treated with tested dose of the Fe$_2$O$_3$@PFDH NCs and saline.
Figure S18. Hematology data of the mice treated with saline and Fe$_2$O$_3$@PFDH.