Support information

A Novel Theranostic Nanoplatform (PB@FePt-HA-g-PEG) for Tumor Chemodynamic-Photothermal Co-Therapy and Triple-imaging (MR/CT/PI) Diagnosis

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Part I. Supplemental Experimental Methods

1. Experimental section

Platinum(II) 4-pentanedione, iron(III) 4-pentanedione, $FeCl_3.6H_2O$, $K_4[Fe(CN)_6].3H_2O$, Cysteamine, Ethylene glycol and citric acid were obtained from Aladdin (Shanghai, China) and used as received. Hyaluronic acid (MW=10~15 kDa) was received from Freda Biochem (Shandong, China). NH_2-PEG (MW=5 kDa) was purchased from Ponsure Biotechnology (Shanghai, China). Aqueous solutions were prepared with deionized water (18.25 MU cm) produced from a Milli-Q water purification system.

1.1. Preparation of Prussian blue nanocubes (PB NCs)

PB NCs were prepared according to the previous literature. Typically, 0.5 mmol citric acid was added into 20 mL $FeCl_3$ aqueous solution (1.0 mM) under stirring at 60 ℃. Then, 20 mL $K_4[Fe(CN)_6]$ aqueous solution (1.0 mM) containing 0.5 mmol citric acid was added dropwisely into the above solution under stirring at 60 ℃. A clear bright blue dispersion formed immediately during the mixing process, the mixture was cooled down to room temperature with stirring for another 30 min. To collect as-made PB NCs, 40 mL acetone was added into the dispersion. The mixture was then centrifuged at 14,800 rpm for 15 min to collect PB NCs, then PB NCs were washed with acetone for three times. Finally, the obtained PB NCs were dissolved in water for future use.

1.2. Fabrication of the nanocomposites PB@FePt NCs

Nanocomposites PB@FePt NCs were fabricated as described in our previous work. The PB NCs and 0.1 mmol platinum(II) 4-pentanedione together with 0.2 mmol iron(III) 4-pentanedione were introduced into a three-neck flask. The flask was then placed on an IKA magnetic heating stirrer and heated under N_2 atmosphere. The metal ion-polyol solution was refluxed with constant stirring speed and maintained for one hour.

1.3. Preparation of PB@FePt-HA-g-PEG NCs

Nanocomposites PB@FePt-HA-g-PEG NCs were fabricated in two steps.

Preparation of PB@FePt-NH_2 NCs : 20 mg PB@FePt NCs were added into 10 mL cysteamine hydrochloride solution (10 mg mL^{-1}) sonicated for 20 minutes and stirred overnight. The reaction mixture was washed with deionized water several times by ultra-filtration to remove residual cysteamine hydrochloride.

Preparation of PB@FePt-HA NCs: HA-PEG (50 mg) was dissolved in 5 mL of phosphate-buffered saline (PBS, pH 7.4) followed by addition of 24 mg of EDC·HCl and 18 mg of NHS. After 1 h of reaction, the activated HA was added dropwise into 5 mL PB@FePt-NH_2 NCs in PBS (5mg mL^{-1}) and stirred overnight. The reaction mixture was washed with deionized water several times by ultra-filtration to remove residual EDC, NHS.

Preparation of PB@FePt-HA-g-PEG NCs: 50 mg PB@FePt-HA NCs was resuspended in PBS solution followed by addition of 24 mg of EDC·HCl and 18 mg of NHS. After 1 h of reaction, NH_2-PEG (80 mg) was added into the solution and stirred overnight.

Preparation of PB@FePt-HA-g-PEG/FITC NCs: the same operating steps as the obtain PB@FePt-HA-g-PEG NCs while NH_2-PEG (80 mg) was replaced by NH_2-PEG/FITC. After stirred overnight, the reaction mixture was washed with deionized water several times by ultra-filtration to remove residual EDC, NHS.

1.4 Calculation of the Photothermal Conversion Efficiency

The photothermal conversion efficiency (η) can be acquired from the data (Temperature change of of PB@FePt-HA-g-PEG NPs ([PB@FePt-HA-g-PEG] = 4 mg·mL^{-1}) under one cycle) in Figure S5 according to following equation[1].

$$\eta = \frac{hS(T_{max} - T_{surr}) - Q_{dis}}{I(1 - 10^{-A_{808}})} \quad (1)$$

Where h is the heat transfer coefficient, S is the surface area of the container, T_{max} is the equilibrium temperature, T_{surr} is the ambient temperature, Q_{dis} is the heat dissipated from light absorbed by the container itself, I is the laser power used in the experiment, A_{808} is the absorbance of PB@FePt NCs solution at 808 nm. T_{max}=60.7°C, T_{surr}=20.9°C, I=1.5 W/cm^2, A_{808}=1.35, Q_{dis} = (5.4◊10^{-4}) I=0.95 mW.

The value of hS can be obtained using the following equation.

$$hS = \frac{m_W C_W}{\tau_s} \quad (2)$$

where m_w is the mass of water, C_w is the heat capacity of water, τ_s is the time constant of the sample system. m_w=1.8 g, C_w=4.2 J/g.

To determine τs, a dimensionless driving force temperature θ is introduced, which is defined by the following equation.

$$\theta = \frac{T - T_{surr}}{T_{max} - T_{surr}} \quad (3)$$

$$t = \tau_s (- ln\theta) \quad (4)$$

Where T is the real-time temperature of the sample when the laser was turned off, t represents time. As can be seen from Figure S5, τs is determined to be 216.19 s.

1.5 Calculation of Cell Viabilities

The WST-1 assay was measured with a Biotek Elx 800 Microplate Reader. The cell viabilities were all calculated using the following equation (5):

$$\% \text{ cell viability} = \frac{\Delta \text{ Csamples} - \Delta \text{ Cblack}}{\Delta \text{ Ccontrol} - \Delta \text{ Cblack}} \times 100\% \quad (5)$$

Where $C_{samples}$ was obtained in present of PB@FePt-HA-g-PEG NPs, $C_{control}$ was obtained in absent of PB@FePt-HA-g-PEG NPs and C_{black} was obtained in the absent

of both PB@FePt-HA-g-PEG NPs and cells. Δ means the difference value before and after the addition of WST-1.

1.6 The Construction of Tumor-Bearing Mice Model

Female Balb/c mice were purchased form Jinan Peng Yue Experimental Animal Breeding Co,. Lid. and used under protocols approved of Qilu Hospital Laboratory Animal Center (accreditation number: GLP080030008, State Food and Drug Administration). The 4T1 tumor-bearing Balb/c mice model was generated with volume of 100 mm^3 by subcutaneous injection of 2×10^5 cells of mice and growth for 14 days.

2. Characterization

The phase and crystallography of the products were characterized by using a D8 Advance powder system using Cu Ka radiation (k = 0.1542 nm, 40 kV, 20 mA). Transmission electron microscopy (TEM) images of the nanoparticles were obtained using a JEOL 2100 transmission electron microscope at an acceleration voltage of 200 kV. UV-vis-NIR spectra were obtained with a Shimadzu UV-1800 spectrophotometer. The size and the Zeta potentials of nanoparticles were measured using NANO ZS (Malvern, USA).

2.1. Cell labeling and microscopy imaging

MCF-7 human breast cancer cells were cultured in the standard cell medium recommended by American type culture collection (ATCC), under 37 ℃ within 5% CO_2 atmosphere. Cells seeded into 96 well plates were incubated with different concentrations of PB@FePt-HA-g-PEG NCs for 24 h. Relative cell viabilities were determined by the standard WST-8 assay. For in vitro photothermal therapy, MCF-7 cells were incubated with different concentrations of PB@FePt-HA-g-PEG NCs for 6 h and then irradiated by an 808 nm laser at the power density of 1 W/cm^2 for 10 min.

2.2. In vivo Anticancer Therapy

Mice were subcutaneously injected with wild-type 4T1 cells (1×10^5 cells). Once tumors reached an approximate size of 100 mm^3, mice were randomly divided into eight groups with four mice in each group. Then, mice were intratumor injected with 50 μL of saline, PB@HA-g-PEG, FePt@HA-g-PEG, PB@FePt-HA-g-PEG (with the same concentration of nanoparticles, 2mg mL^{-1}) on the first day. The other four groups were corresponding injected with above nanomaterials and 30 min later the mice were illuminated under the laser light (808 nm laser, 1.5 W cm^{-2}) for 5 min. Twenty-four hours after the injection, tumors were Tumor size and mice weight were measured immediately before the injection. Tumor volume was defined as $V = W^2 \times L/2$, where W and L are the shortest and longest diameters of tumors, respectively. Relative tumor volume was defined as V/V0 (V0 is the tumor volume when the treatment was initiated). After mice were sacrificed, H&E staining were carried out.

Part II. Supplemental Figures

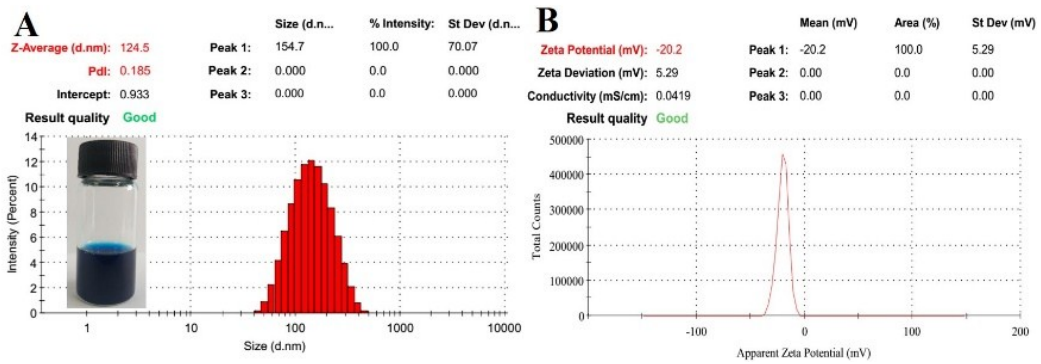

Figure S1. (A) The DLS of PB NCs, the inset is the picture of PB NCs solution ; (B) The zeta potential of PB NCs.

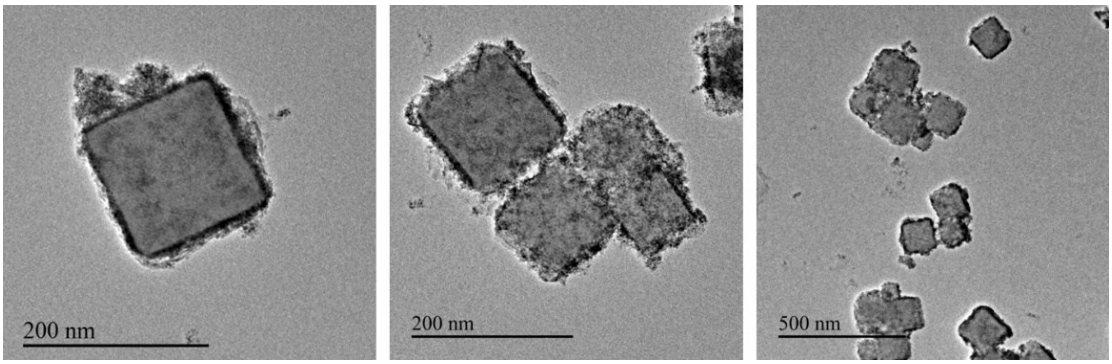

Figure S2. (A) The TEM image of PB@FePt-HA-g-PEG ; (B-C) The enlarged view of PB@FePt-HA-g-PEG NCs.

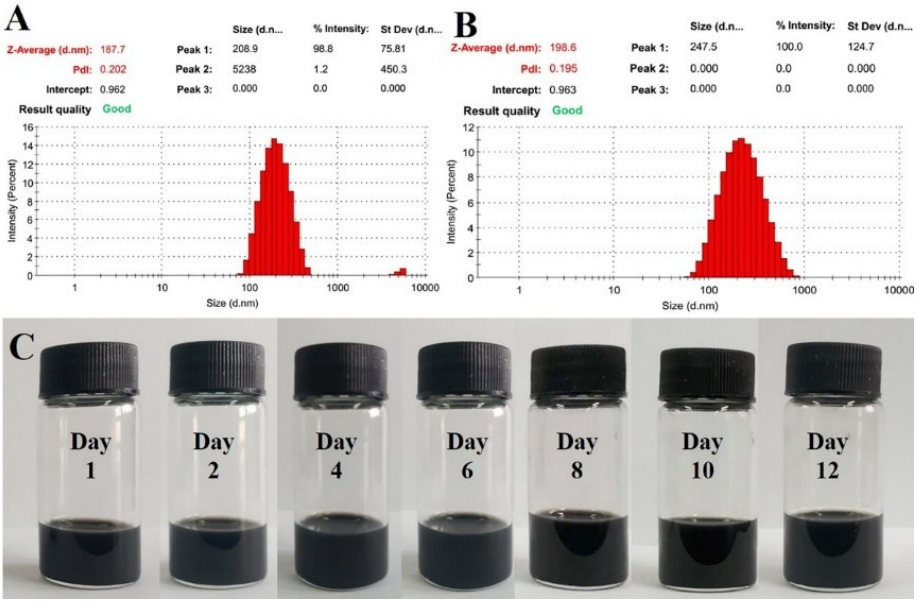

Figure S3. The DLS of the PB@FePt NCs solution at the first day; (B) The DLS of the

PB@FePt NCs solution at the twelfth day; (C) The pictures of the PB@FePt NCs solutions from the first day to the twelfth day.

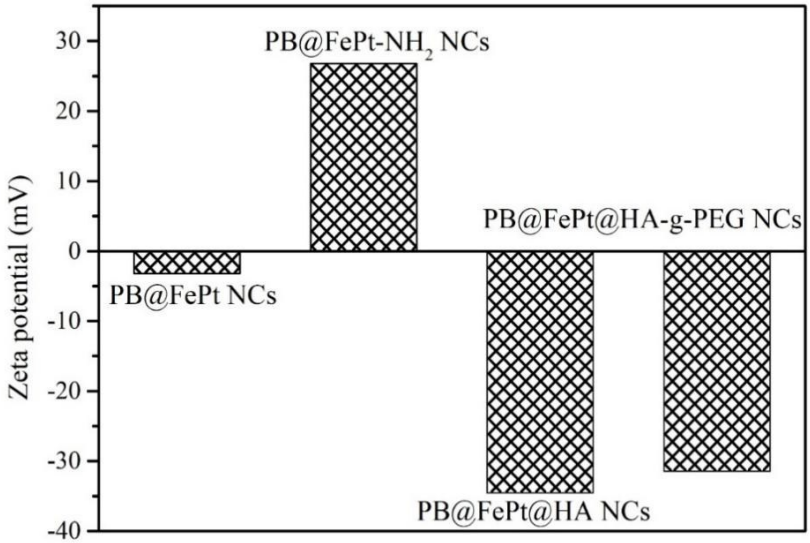

Figure S4. The zeta potentials of PB@FePt NCs, PB@FePt-NH_2 NCs, PB@FePt-HA NCs and PB@FePt-HA-g-PEG NCs.

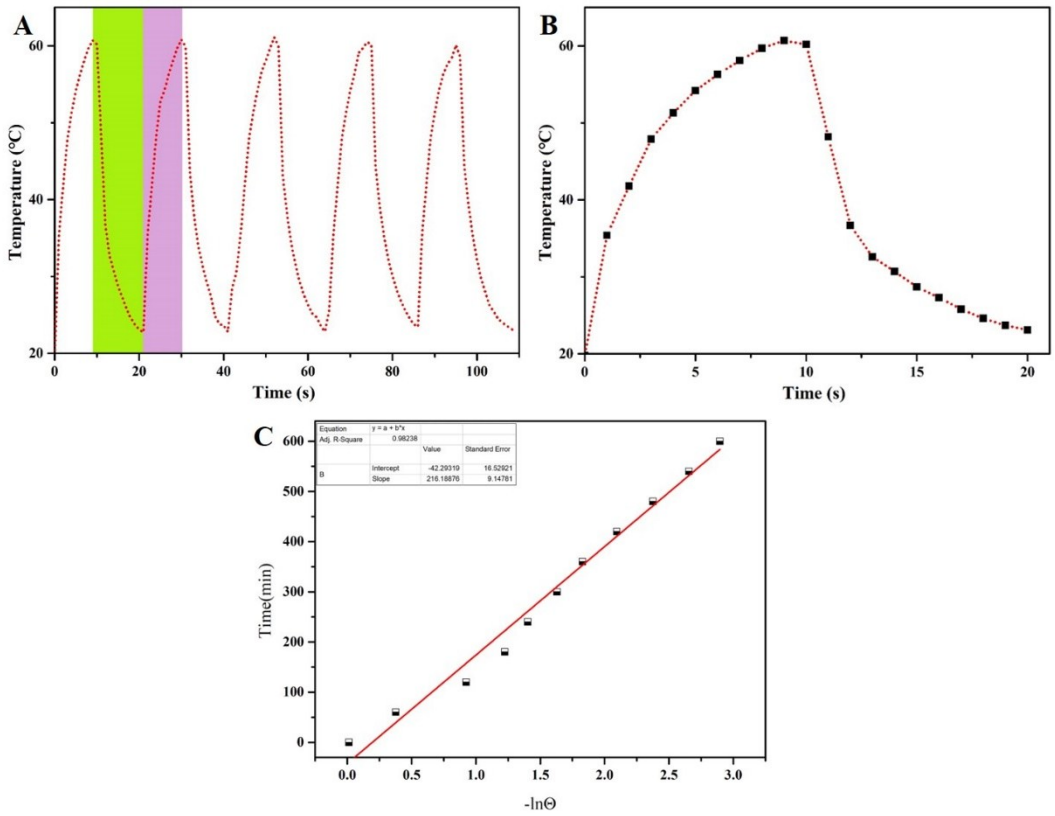

Figure S5. (A) The photothermal conversion stability of PB@FePt NCs ([PB@FePt NCs] = 1 mg·mL^{-1}) responded to 808 nm laser irradiation (1.5 W/cm^2) for five cycles.

(B)Temperature change of of PB@FePt NCs under one cycle. (C) Linear time data versus-lnθ obtained from a cooling period.

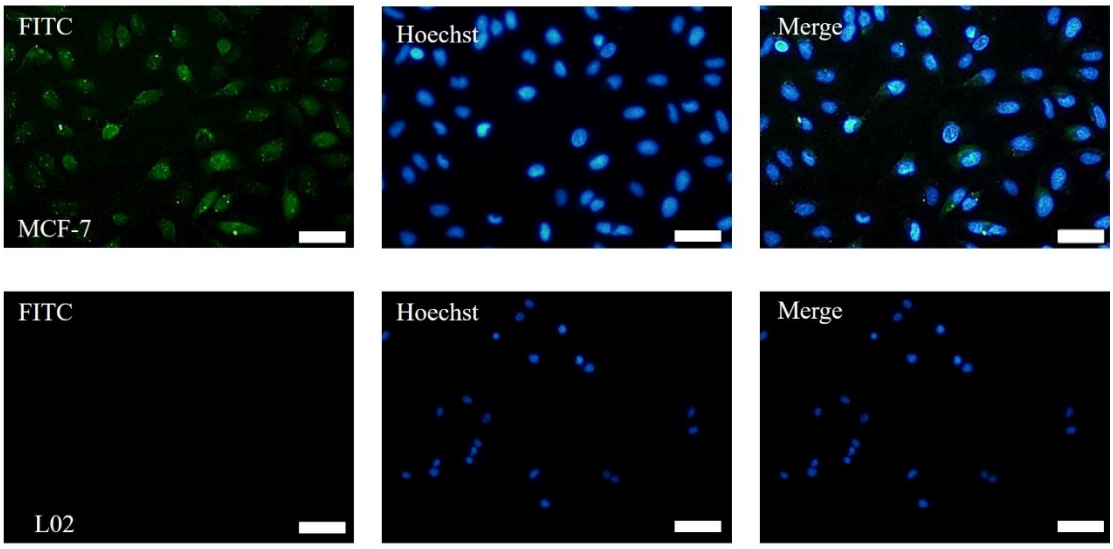

Figure S6. The fluorescence microscope pictures of L02 (A) and MCF-7 cells (B) incubating with PB@FePt-HA-g-PEG/FITC (scale bars: 200 μm).

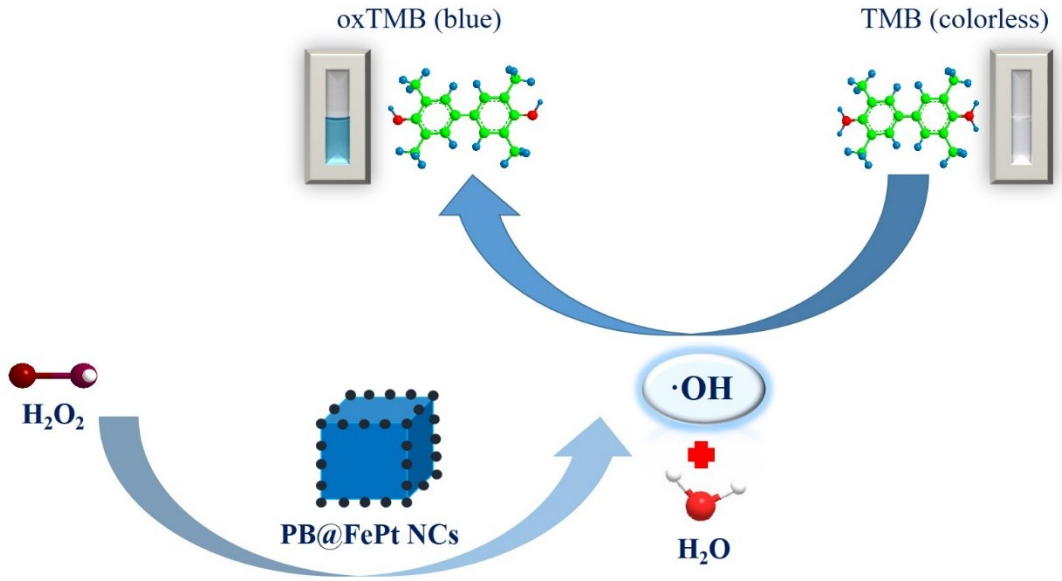

Figure S7. The catalytic mechanism of obtained PB@FePt NCs catalyze H_2O_2 into hydroxyl radical (▪OH)[2].

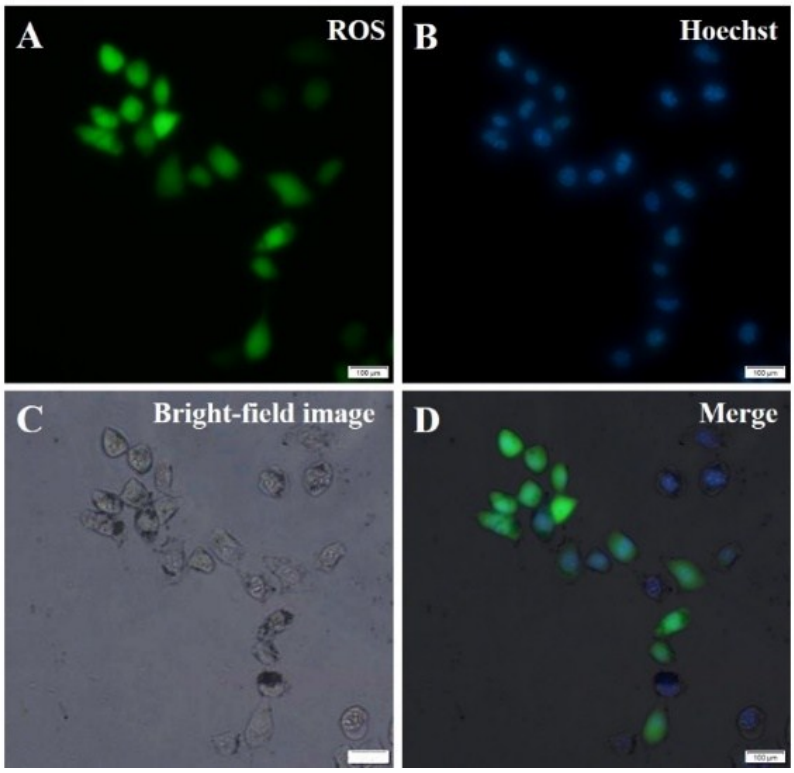

Figure S8. (A) Fluorescent images of DCFH-DA (for ROS), Hoechst 33342 (for cell nucleus) and bright-field images of MCF-7 cells.

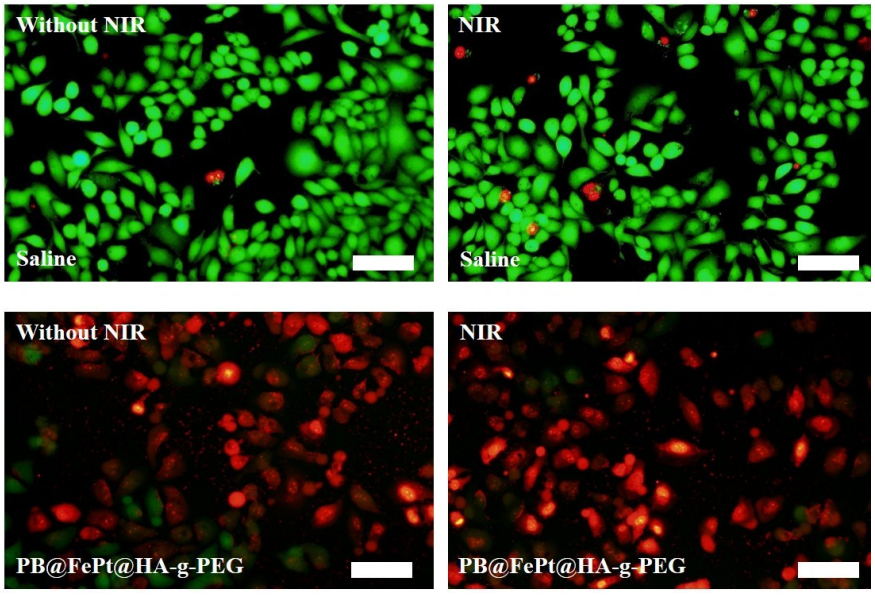

Figure S9. Fluorescent images of 4T1 cells co-stained by Calcein-AM (green fluorescence, live cells) and PI (red fluorescence, dead cells) of different treatments (scale bars: 200 μm).

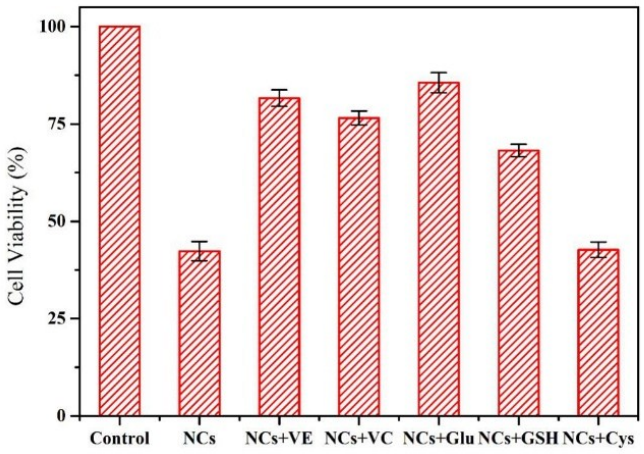

Figure S10. Cell viabilities of MCF-7 cells processed with PB@FePt-HA-g-PEG NCs (abbreviate as NCs), NCs + VE, NCs + VC, NCs + Glu, NCs + GSH and NCs + Cys.

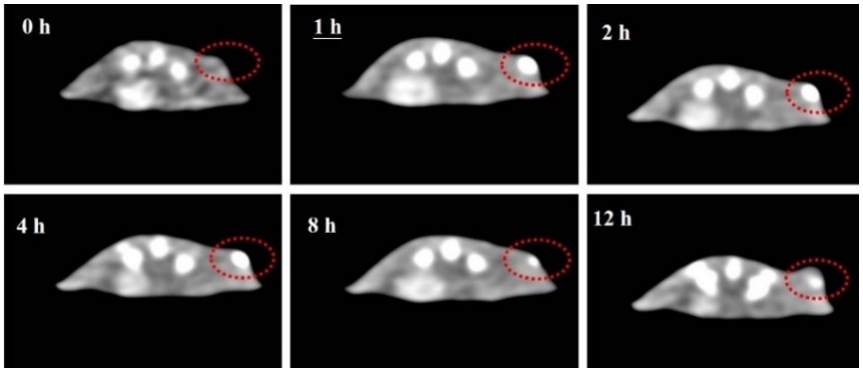

Figure S11. The CT images of tumor-bearing mouse after treated with PB@FePt-HA-g-PEG NCs for 12 hours.

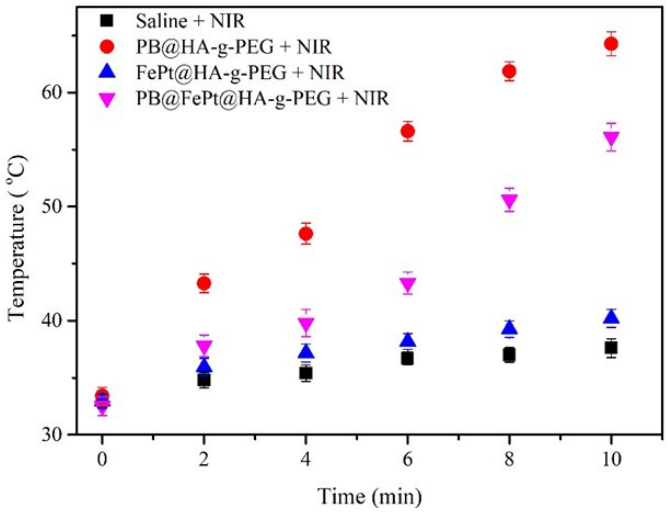

Figure S12. The photothermal heating curves of the photothermal therapy of the tumor bearing mouses.

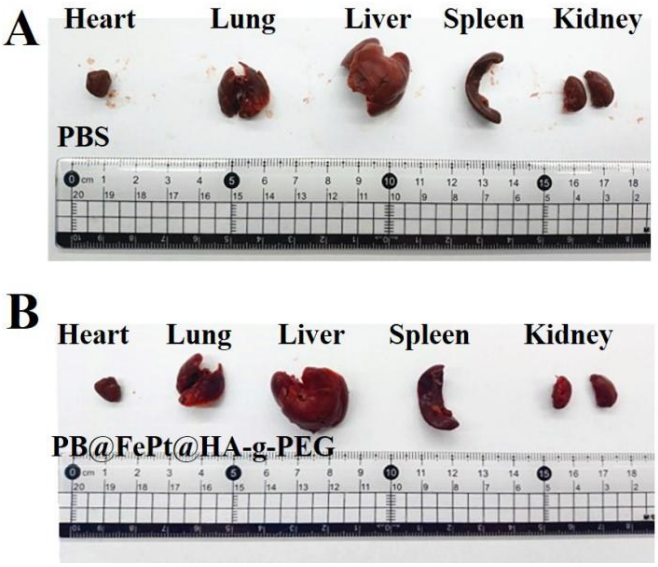

Figure S13. The images of the main organs of the saline control groups and the experimental groups.

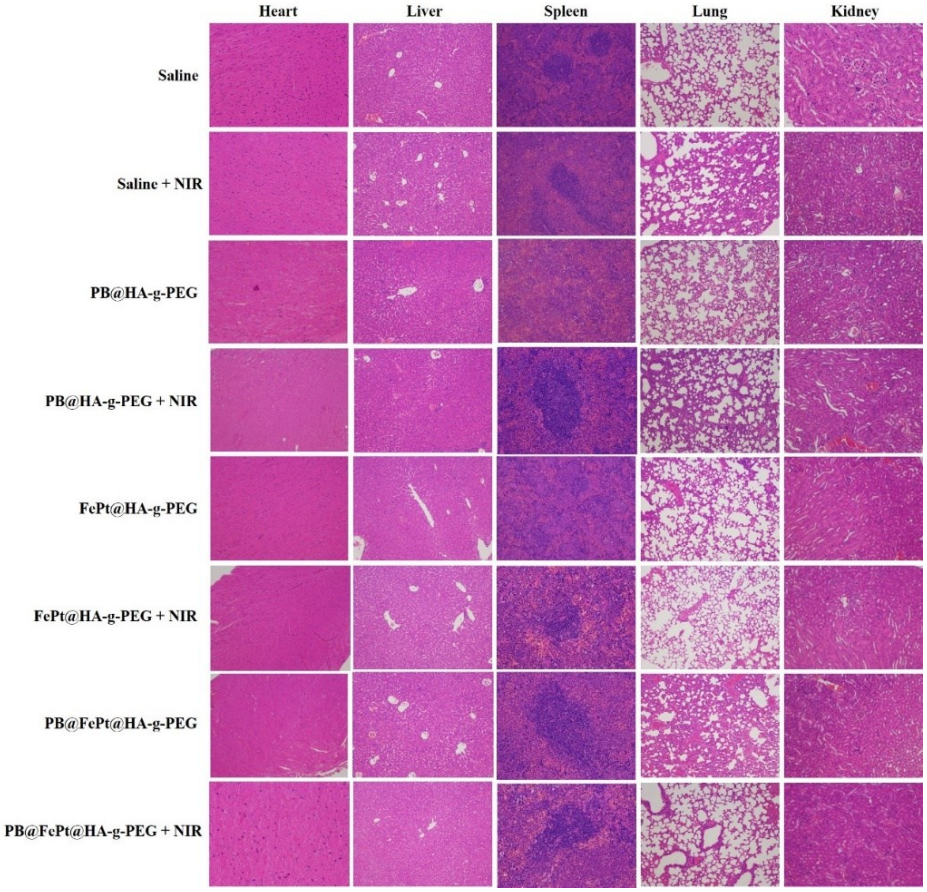

Figure S14. The images of H&E stained main organs slices after 16-day treatment with nano-materials.

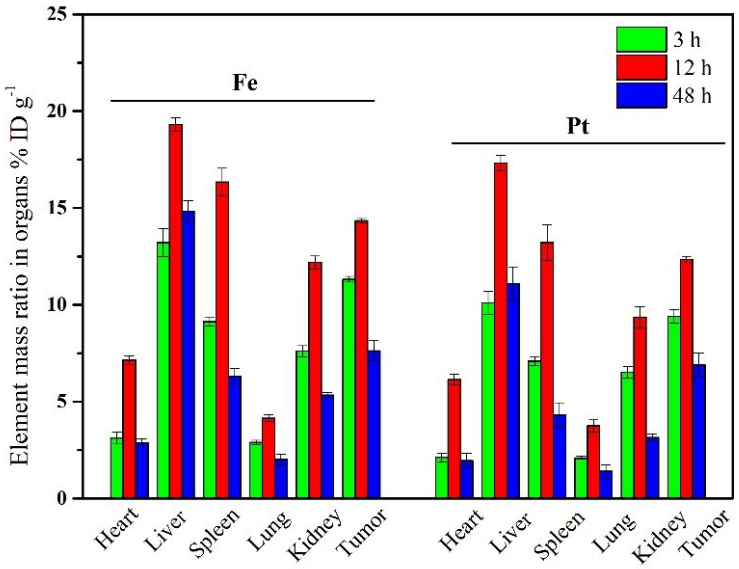

Figure S15. The biodistribution of Fe and Pt in tumors and major organs measured by ICP-MS after intravenous injection with PB@FePt@HA-g-PEG for 3, 12 and 48 h.

Part IV. Supplemental References

1. Zong, S.; Wang, L.; Yang, Z.; Wang, H.; Wang, Z.; Cui, Y., Black Phosphorus-Based Drug Nanocarrier for Targeted and Synergetic Chemophotothermal Therapy of Acute Lymphoblastic Leukemia. *ACS Appl Mater Interfaces* **2019,** *11* (6), 5896-5902.

2. Hu, Z.; Dai, Z.; Hu, X.; Chen, K.; Gao, C.; Zheng, X.; Yu, Y., Synthesis of PB@FePt hybrid nanoparticles with peroxidase-mimicking activity for colorimetric determination of hydrogen peroxide in living cells. *Anal. Methods* **2019,** *11* (5), 677-683.