Supplementary Information

Biomineralized iron oxide-polydopamine hybrid nanodots for contrast-enhanced T_1-weighted magnetic resonance imaging and photothermal tumor ablation†

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Experimental Section

Materials

Human serum albumin (HSA) (Beijing wokai biotechnology Co. Ltd.). Dopamine hydrochloride (Shanghai alighting biochemical technology Co. LtD.). Potassium ferrate (Shanghai Mclean Biotechnology Co. Ltd.). Tris (Shanghai Bioengineering Co. Ltd.). Dimethyl sulfoxide (Sinopharmaceuticals group chemical reagent Co. Ltd.). Fetal bovine serum (Hyclone). The common chemical reagents are pure analytical reagents, and the experimental water is deionized water. Carbon supporting membrane (Beijing xinxing bairui technology Co. Ltd.). Ultrafiltration tube (MWCO 100 kDa, Millipore Co, USA). Filter membrane (220 nm, 800 nm).

Synthesis

The IO/PDA hybrid nanodots were synthesized by one pot method using human serum albumin (HSA). First, 100 mg dopamine (DA) and 200 mg HSA were dissolved in 100 ml pH 8.5 Tris buffer. Then 2.0 mL of 15 mg mL^{-1} K_2FeO_4 was added drop by drop to the mixture of DA and HSA, followed by 2 hours vigorous stirring at room temperature to generate IO/PDA-NDs. Over time, the colorless solution turned dark brown. A small amount of sediment was removed using an 800 nm needle filter membrane and the obtained products were centrifuged through an ultrafiltration (100 kDa, Millipore) to purification. By changing the mass ratios of DA, HSA and K_2FeO_4, various IO/PDA-NDs were synthesized by the similar way.

Characterization

The morphology of the IO/PDA-NDs was observed using a transmission electron microscope (TEM, Hitachi H-600) at 120 kV. The absorption spectrum of IO/PDA-NDs was measured by UV-visible spectrophotometer (Shimadzu UV2600). Fe was quantitatively analyzed by atomic absorption spectrometer (AA240FS-GTA120).

Chemical stability, and photostability

The chemical stability of IO/PDA-NDs aqueous solution was determined by UV-vis absorbance at 785 nm. The absorption spectra of IO/PDA-NDs were monitored in deionized water, 0.9 % NaCl, PBS at pH 5.0, PBS at pH 7.4, RPMI 1640 medium with 10 % serum or not at 0, 2, 4, 8, 12, and 24 h, respectively. To evaluate the photostability, IO/PDA-NDs were irradiated under 785 nm (0.5 $W.cm^{-2}$) for 0, 0.5, 1, 2, 4, 6, 10 and 15 min, respectively. Then, the UV-Vis absorbance at 785 nm was recorded by UV-visible spectrophotometer.

Photothermal effect

The illumination is performed by the laser of 785 nm with power intensity of 0.5 $W\ cm^{-2}$. To study the photothermal effects of IO/PDA-NDs, 0.5 mL IO/PDA-NDs were irradiated at different Fe concentrations ranging from 0.05 mM to 0.5 mM for 5 min. A digital temperature sensor was used to monitor the temperature of the solution every 30 seconds. The temperature change (ΔT) was used to evaluate the photothermal effect. To evaluate the photothermal conversion efficiency, 0.5 mL IO/PDA-NDs at a concentration of 0.5 mM Fe were irradiated for 12 min, and then the sample was cooled to room temperature by turning off the laser. During this process, the temperature of

the solution was recorded with a thermometer every 30 seconds.

In vitro MR imaging

The IO/PDA-NDs aqueous solution was gradient diluted to various concentrations including 0.03125 mM, 0.0625 mM, 0.125 mM, 0.25 mM, 0.5 mM, and MR mappings were performed on 3.0 T clinical magnetic resonance imaging system (Philips, Achieva). The relaxivity value (r_1) of IO/PDA-NDs was calculated according to the reference through the concentration of Fe and the reciprocal of relaxation time.

Cellular uptake and endocytic pathway

4T1 cells at logarithmic growth stage were collected and inoculated in 6-well cell culture plates with a density of 2×10^5 cells per well. After 24 hours, IO/PDA-NDs at the concentration of 0.1 mM (measured in Fe) were added to each well for another 0.5 h, 2 h, 6 h and 12 h incubation. At the corresponding time point, trypsin was used for digestion and the cell count board was used to count the number of cells in each well. The cells were then crushed with an probe sonicator, followed by digestion with 100 μL of concentrated nitric acid. Finally the content of the metallic element iron in the lysate was determined by an atomic absorption spectrometer. For endocytic pathway study, 4T1 cells at logarithmic growth stage were collected and inoculated in 6-well cell culture plates with a density of 2×10^5 cells per well. After 24 h, cells was respectively treated with 200 μL of various inhibitors, including chlorpromazine (10 μg mL^{-1}), filipin (5.0 μg mL^{-1}), amiloride (100 μg mL^{-1}) at 37 °C or 4 °C without inhibitors for 1 h incubation. After 1 hour, IO/PDA-NDs at the concentration of 0.1

mM (measured in Fe) were added into cells for another 2 hours incubation, followed by washing using PBS for 3 times and analyzing Fe concentration in cells.

Intracellular distribution

4T1 cells at logarithmic growth stage were collected and seeded in 20 mm glass-bottom dish at the density of 1×10^5 cells per well for 24 h incubation. Then rhodamine B-labelled IO/PDA-NDs at the concentration of 0.1 mM (measured in Fe) were added for 4 h incubation at 37 ℃. The medium containing the IO/PDA-NDs was discarded and washed with PBS solution for 3 times. Subsequently, 1.0 mL of 5 μg mL^{-1} Hoechst 33342 dye was added into the cells for 10 min incubation at 37 ℃. And then 1.0 mL of 50 μM Lysotracker Green DND-26 dye was added for 3 min incubation at 37 ℃. Finally, the cells were washed using PBS for 3 times, followed by adding 4 % of 1.0 mL paraformaldehyde with 15 min incubation. The intracellular distribution of IO/PDA-NDs was observed by confocal laser scanning microscope (CLSM, Zeiss LSM710).

Cytotoxicity

MTT assay was used to investigate the cytotoxicity of IO/PDA-NDs on 4T1 cells. 4T1 cells at logarithmic growth stage were collected and inoculated into 96-well plates at a density of 5×10^3 cells per well for 24 h incubation. IO/PDA-NDs at different concentrations of 0, 10, 50, 100, 200 and 300 μM (measured in Fe) were added into each well and co-incubated with cells for 24 h. Then the cells were washed 3 times with PBS, followed by replacement of fresh culture medium. After another 24 h incubation,

the cell viability was evaluated using MTT assay by measuring absorbance value (OD value) at 490 nm. The cell viability was calculated by the following formula:

$$\text{Cell viability (\%)} = (OD_{\text{IO/PDA-NDs}} / OD_{\text{control}}) \times 100\%$$

Biodistribution

The biodistribution of IO/PDA-NDs was studied on 4T1 tumor bearing mice by intravenous injection of IO/PDA-NDs at the dose of 20.0 μmol kg^{-1} Fe (n = 3). After 24 hours of administration, the mice were sacrificed and their heart, liver, spleen, lung, kidney and tumor tissues were extracted, weighed and digested. The quantification of Fe was determined by an atomic absorption spectrometer.

***In vivo* MR imaging**

For *in vivo* MR imaging, IO/PDA-NDs (20.0 μmol kg^{-1} Fe) were intravenously injected into the 4T1 tumor bearing mice. The T_1-weight images were obtained at 0, 2, 6, 12, 24 h after injection using a 3.0 T clinical MR imaging system (Philips, Achieva) with specially designed animal imaging coils. The T_1-weighted MR signal intensity was collected from the selected region of interest in the MR image, and then the SNR (Signal/Noise Ratio) was calculated. T_1-weight images were obtained at a 3.0 T MR imaging system (Philips, Achieva) with a fat-saturated 3D gradient echo imaging sequence (TR/TE = 400/10 ms, 256×256 matrices, slices = 3, thickness = 2 mm, averages = 4, FOV = 60×60).

***In vivo* infrared thermography**

To evaluate the in vivo hyperthermia at tumor, IO/PDA-NDs were intravenously

injected into the 4T1 tumor bearing mice at the dose of 20.0 μmol kg^{-1} Fe. The intravenous PBS served as the control group. 24 h later, 785-nm laser at the intensity of 0.5 W cm^{-2} was applied to irradiate the tumor for 5 min. The temperature of tumor was monitored using an infrared camera (FLIR E50).

Antitumor efficacy

The antitumor efficacy of IO/PDA-NDs was tested in mice bearing 4T1 subcutaneous tumors. When the tumor volume was about 70 mm^3, the mice were randomly divided into five groups (n = 5): (1) PBS; (2) PBS/Irradiation (785 nm, 1.5 W cm^{-2}, 5 min); (3) IO/PDA-NDs (20.0 μmol kg^{-1} Fe); (4) IO/PDA-NDs (20.0 μmol kg^{-1} Fe) /Irradiation (785 nm, 1.5 W cm^{-2}, 5 min); (5) IO/PDA-NDs (20.0 μmol kg^{-1} Fe) /Irradiation (785 nm, 0.5 W cm^{-2}, 5 min). At 24 hours post-injection, the tumors in the light group were exposed to the laser for 5 minutes. The tumor volumes were monitored during the following 30 days according to the equation of $V= L\times W^2/2$, where W and L are the tumor measurements at the widest and longest dimensions, respectively. The relative tumor volume was analyzed with V/V0 (where V0 was the tumor volume before treatment) to present the antitumor efficacy. The weight of mice was also monitored during the treatment.

Histological staining

Hematoxylin and eosin (H&E) staining was performed to validate the *in vivo* photothermal damage of IO/PDA-NDs. IO/PDA-NDs were intravenously injected into the mice at the dose of 20.0 μmol kg^{-1} Fe, followed by 5 min irradiation (785 nm, 1.5

W cm^{-2}) at the tumors at 24 h post-injection. 6 hours later, major tissues including heart, liver, spleen, lung, kidney and tumor were obtained and fixed with 4 % paraformaldehyde at room temperature. H&E staining was carried out, and the sections were observed using an IX73 bright field microscopy (Olympus).

Figures

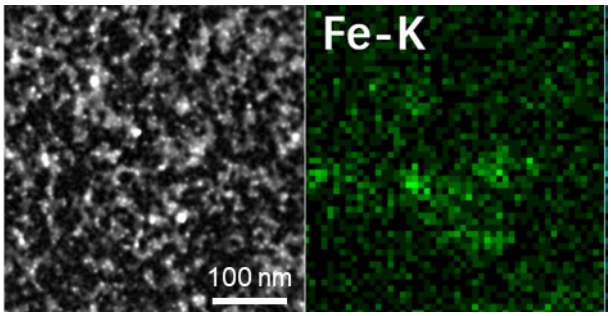

Figure S1. FETEM images and elemental mapping images of IO/PDA-NDs

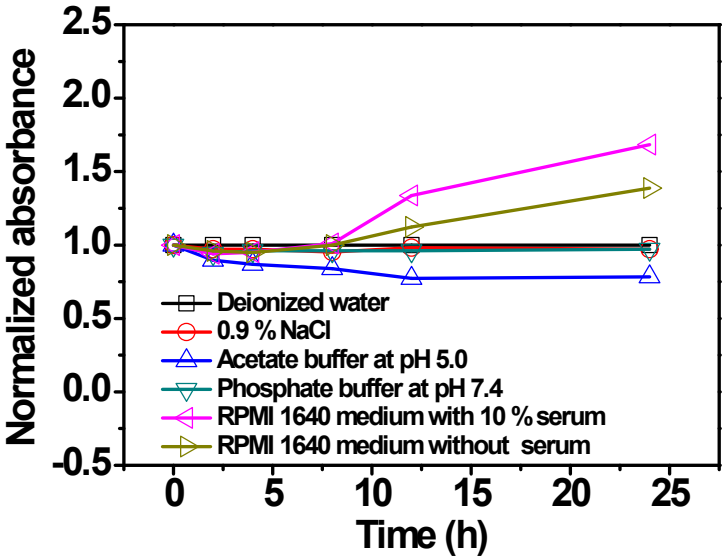

Figure S2. Relative absorbance of IO/PDA-NDs in various solvents for different time

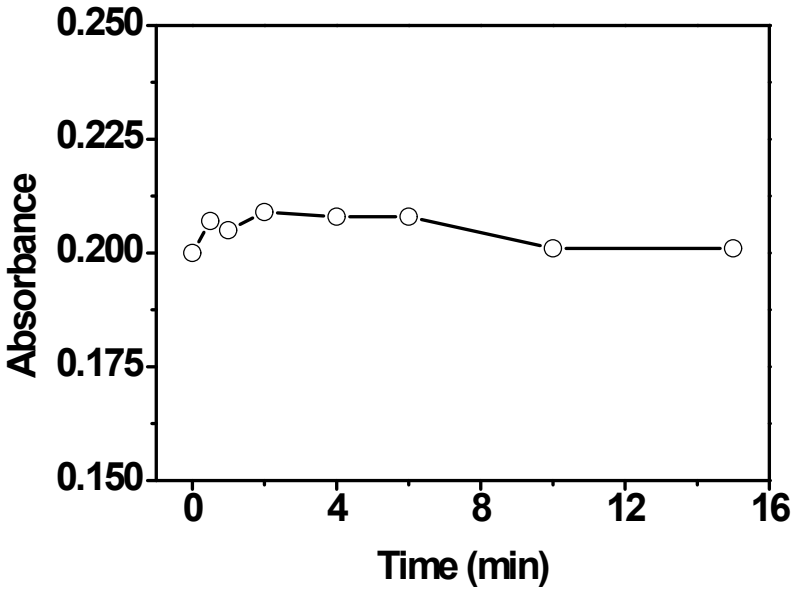

Figure S3. UV-Vis absorbance of IO/PDA-NDs under 0.5 W cm^{-2} irradiation (785 nm) for different time

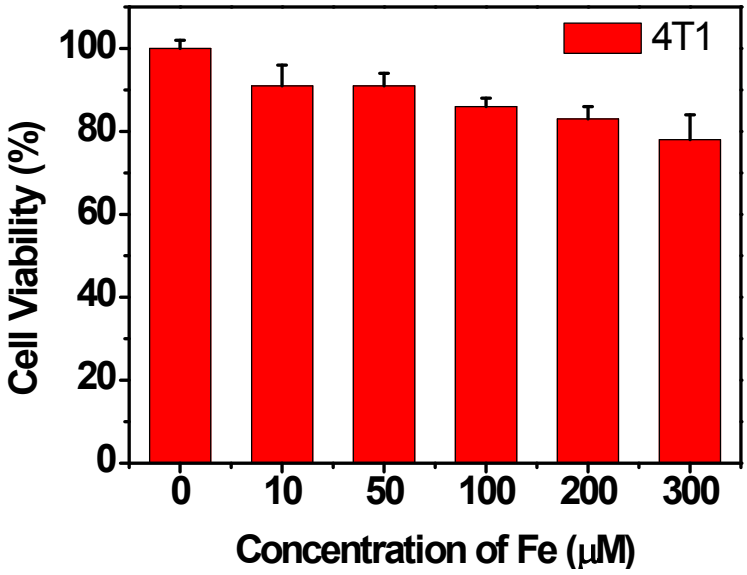

Figure S4. Cell viability of 4T1 cells exposed to different concentrations of IO/PDA-NDs for 24 h

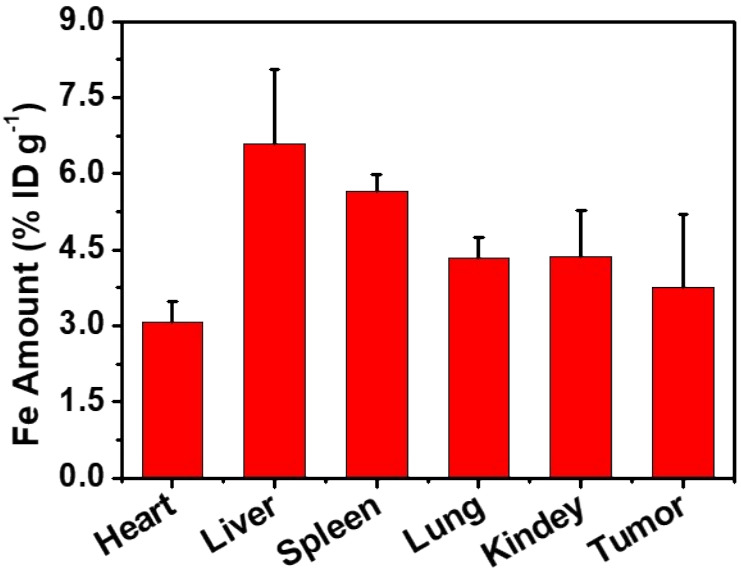

Figure S5. Biodistribution of Fe at various tissues of mice treated with IO/PDA-NDs at 24 h post-injection at the dose of 20 μmol kg^{-1} Fe

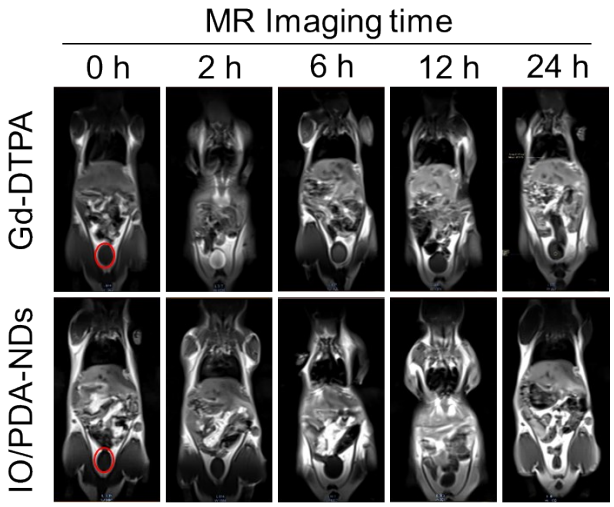

Figure S6. *In vivo* Coronal MR imaging of the mice injected with IO/PDA-NDs and Gd-DTPA at 0, 2, 6, 12, 24 h post-injection, respectively

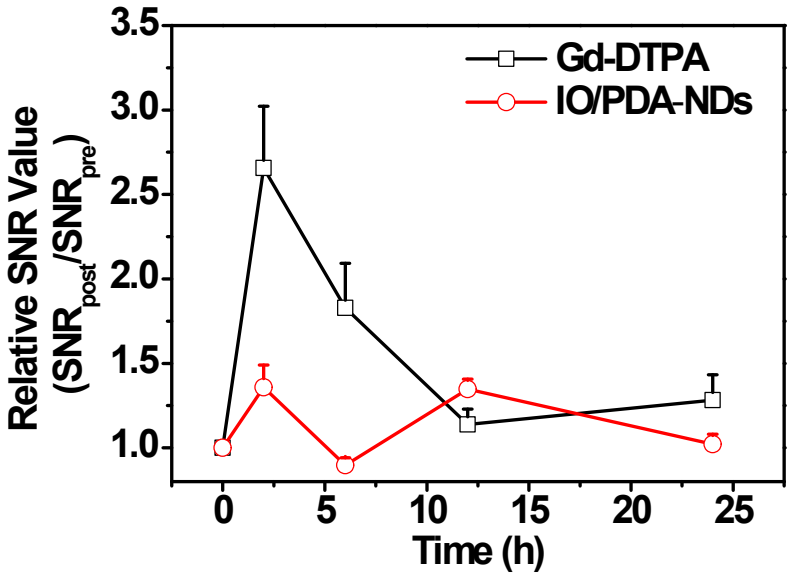

Figure S7. The relative SNR value of the bladder areas from the mice injected with IO/PDA-NDs and Gd-DTPA at 0, 2, 6, 12, 24 h post-injection, respectively