Supporting Information

Pt@polydopamine nanoparticles as nanozymes for enhanced photodynamic and photothermal therapy

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Materials

Dopamine hydrochloride (DA), $NH_3 \cdot H_2O$, H_2O_2 (30%), DPBF were obtained from Sigma-Aldrich Co., indocyanine green (ICG) was obtained from J&K Chemicals. 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) and 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[folate(polyethylene glycol)-2000]-Folate (DSPE-PEG-folate) were obtained from Avanti. Texas Red-DHPE was obtained from AAT Bioquest. Dulbecco's Modified Eagle Medium (DMEM), trypsin with EDTA, fetal bovine serum (FBS) were purchased from Hyclone, DCFH-DA was purchased from Solarbio. All reagents were used as received.

Pt nanoparticles were synthesized according to the method in literature.[1] Typically, 1 gram $H_2PtCl_6 \cdot 6H_2O$ was dissolved in 50 ml ethylene glycol (EG) to form yellow solution, and about 50 ml NaOH EG solution (0.25 M) was added into above solution under stirring to modify the pH value to 10. After 30 minutes stirring, the mixed solution was heated at 180 ^oC for 3 hours under flowing nitrogen gas to form dark-brown platinum colloid solution, which was ready for use after cooling down to room temperature.

Synthesis of Pt-ICG@PDA nanoparticles

PDA nanoparticles were parepared according to our recent work.[2] 40 mL ethanol, 90 mL water, and 2.7 mL $NH_3 \cdot H_2O$ (28-30%) were added and stirred evenly in a flask. After that 500 mg DA dissolved with 10 mL H_2O was added in the above solution, and after a reaction under room temperature for 12 h, the obtained PDA nanoparticles were centrifuged and stored at 4 °C for further use. For the preparation of ICG@PDA, 0.2 mL ICG (1 mg/mL) solution was mixed with 1 mL PDA (0.5 mg/mL), the pH value was adjusted to 2-3, and then vibrated for 4 h. Then 50 μL Pt nanoparticles (1.35 mg/mL) was added and vibrated for another 2 h for Pt-ICG@PDA. The folate was coated on the particle by sonication method. Briefly, 8 mg DMPC and 0.6 mg DSPE-PEG-folate were dissolved by 2 mL methyl alcohol and 2 mL chloroform, then the solution was dried by rotary evaporation for 4 h and hydrated in a water bath of 40 °C and extruded through a 200 nm filter membrane. The above obtained folate solution (4 mg/mL, 0.1 mL) was mixed with 0.5 mg/mL different nanoparticles to obtain PDA, ICG@PDA and Pt-ICG@PDA, all the nanoparticles were collected by centrifugation at 10000 rpm for 10 min.

Apparatus and characterization

The morphology images of PDA, ICG@PDA, Pt-ICG@PDA nanoparticles were collected by transmission electron microscopy (Hitachi, 7700). The Texas Red dyed Pt-ICG@PDA was obtained by confocal laser scanning microscopy (Olympus, FV1000). UV-vis absorbance spectra were obtained by a spectrophotometer (SHIMADZU, 2600). The quantification of loaded Pt on the nanoparticles was detected by ICP-MS (Thermer Fisher, iCAP RQ). Values of 96 wells plate for cytotoxicity detection were recorded by a microplate reader (PE Enspire). Photoacoustic studies of samples and animals were all conducted by iThera instrument, MSOT invision 128. Temprature changes of samples were recorded by Thermal Imager (FLIR, E4). Fluorescent imaging of small animal was performed with a multispectral small-animal imaging system (PerkinElmer, IVIS Spectrum).

Detection of multimodal imaging in solution

Firstly, the photoacoustic performace of ICG@PDA was conducted, and the signals were monitored at 700, 730, 760, 800 and 850 nm, the images and intensities were presented in the combination mode of all the detected wavelengths. The concentrations of detected samples were 0.25, 0.5, 0.75, 1 (mg/mL), respectively. For the photothermal properties, particles (PDA and ICG@PDA) were irradiated by a 808 nm laser of 1.13 W (4 W/cm^2) for 11 min when the temprature reached the maximum point, and then the light was shut off. The temprature was recorded every 20 s and pure water without sample was the control group.

The ROS generation ability of nanoparticles

The ROS detection reagent, DPBF, was used in this experiment. Different samples of the same concentration (H_2O, H_2O_2, ICG@PDA + H_2O_2, Pt-ICG@PDA + H_2O_2) were mixed with 0.8 mL DPBF (0.025 mg/mL) ethanol solution, the volumes of H_2O_2 were all 30 μL. Then samples were irradiated with 808 nm laser (1.13 W, 6 mm, 4 W/cm^2) for 2 min. The UV absorbance spectra were collected every 20 s under the irradiation, the spectra were recorded from 350 nm to 500 nm. After that the normalised absorbance values of samples were calculated to compare the abilities of ROS generation.

The ROS detection in cells was conducted by DCFH-DA reagent. In detail, MCF-7 cells seeded in confocal dishes were incubated with ICG@PDA and Pt-ICG@PDA nanoparticles, respectively. After an incubation for 6 h, the above dishes were washed for three times and DCFH-DA was added in and the reagent concentration was 10 μM. Pictures were collected by CLSM before and after the irradiation of 808 nm laser for 8 min.

Cytotoxicity assays

MCF-7 cells were provided by National Center for Nanoscience and Technology, they were incubated with 100 μg/mL Pt-ICG@PDA nanoparticles, after 6 h, the solution was discarded and cells were washed with PBS solution. CLSM images were collected to detect the endocytosis effect. Then the laser cytotoxicity of nanoparticle was evaluated. 1×10^4 cells per well were seeded into plate, after an incubation for 24 h, different volumes (10, 15, 20, 25, 30 μl) of 4 mg/mL nanoparticles (folate labelled PDA, ICG@PDA or Pt-ICG@PDA) were added. 6 h later, all the cells were treated with 808 nm laser for 2 min (1.13 W, 4 W/cm^2), then followed by another incubation of 18 h. CCK-8 reagent was used in this detection, and the UV absorbance values were measured by Microplate Reader (PE Enspire). Dark cytotoxicity was investigated under the same condition except without the laser irradiation.

Animals and live subject statement

BALB/c nude mice (female) around 15 g were used in this experiment, and the mice were provided by Charles River Co., Ltd (Beijing, China). All the conductions were according to the guidelines of Institutional Ethical Committee of Animal Experimentation provided by NCNST (National Center for Nanoscience and Technology), Beijing, China. Study participants were informed regarding the aims of the experiment and agreement was acquired. Tumor models were established by

subcutaneous injection of 5×10^6 cells per mouse. Then the mice were applied to the following detection.

***In vivo* imaging detection**

Firstly, the fluorescence imaging detection was conducted by IVIS Spectrum instrument. Animals were injected with Pt-ICG@PDA nanoparticles via tail vein and the fluorescence intensities were obtained after different periods (4, 8, 12, 24, 48 h). Then the photothermal imaging effect was investigated by a Thermal Imager. Briefly, animals injected with different samples (falate labelled PDA, ICG@PDA or Pt-ICG@PDA) of 15 mg/kg for 24 h were exposed under the laser irradiation (4 W/cm^2) for 5 min. Temprature pictures were collected every minute to record the changes. Then photoacoustic imaging effect was also investigated, photoacoustic intensities were obtained before and after the tail intravenous injection of Pt-ICG@PDA for 24 h. Signals were monitored at 700, 730, 760, 800 and 850 nm, the images were shown in a combination mode.

Anti-tumor effect *in vivo*

When the tumor diameter reached about 5 mm, they were divided into five groups: group 1 was the control group, group 2 was laser group which only treated with laser, group 3 was PDA + laser group, group 4 was ICG@PDA + laser group and group 5 was Pt-ICG@PDA + laser group, all the injection doses of nanoparticles were 15 mg/kg, and the laser intensities were 4 W/cm^2 (5 min), the irradiation was conducted 24 h post-injection via tail vein. The weight of mouse was recorded every two days and the tumor volume was calculated by the equation of $V = (\text{length} \times \text{width}^2)/2$.

Statistical analysis

Mean ± standard deviation (SD) was used in the data presentation. Data statistical analyses were conducted by the method of LSD Duncan, one-way ANOVA analyse, $*P$ means statistically significant < 0.05, $**P$ means statistically significant < 0.01.

Supplementary data:

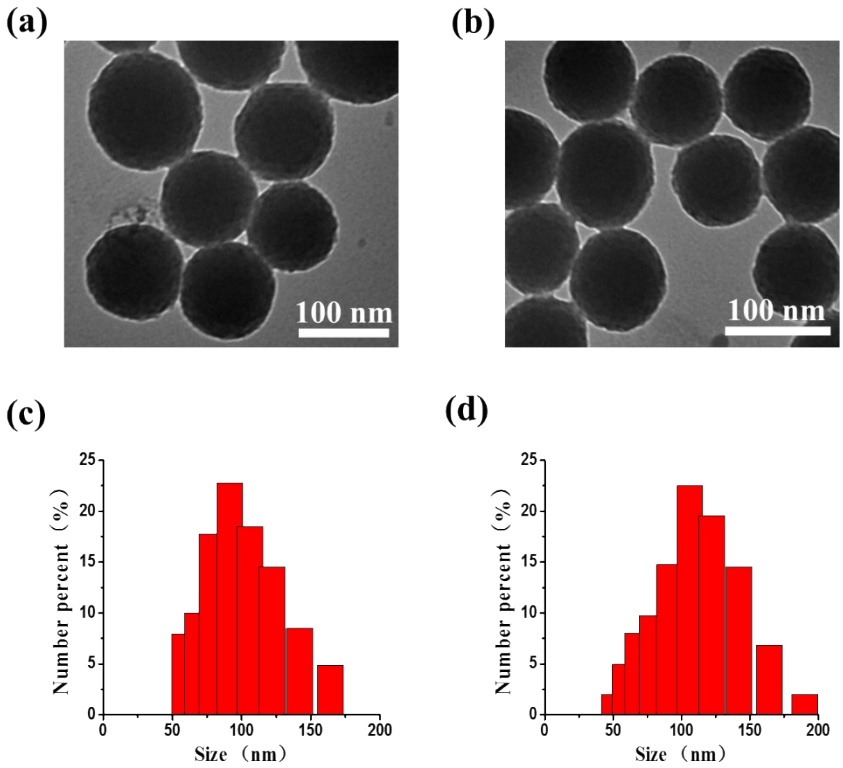

Figure S1. TEM and DLS characterizations of nanoparticles. (a) TEM image of PDA. (b) TEM image of ICG@PDA nanoparticles. (c) DLS result of ICG@PDA naoparticles. (d) DLS result of Pt-ICG@PDA naoparticles.

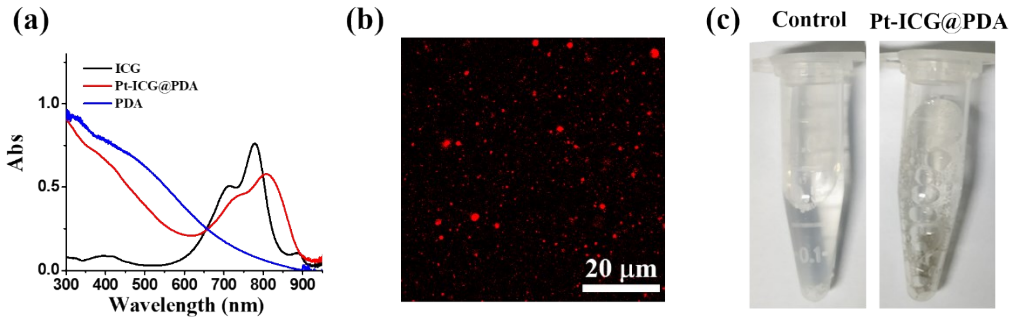

Figure S2. UV and CLSM detection of Pt-ICG@PDA. (a) UV absorbance of ICG, PDA and Pt-ICG@PDA. (b) CLSM image of Pt-ICG@PDA dyed by Texas Red lipid, 559 nm excitation. (c) The images of H_2O_2 solution incubated with/without Pt-ICG@PDA.

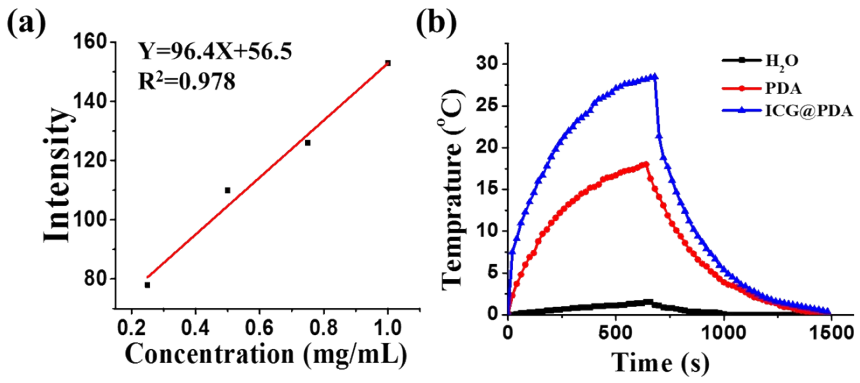

Figure S3. PA and photothermal intensity values. (a) The values of PA indensity of PDAI nanoparticles of different concentrations. (b) Photothermal response of different samples under the irradiation of 808 laser for 11 min when the temperature reached the maximum point and then the light was shut off.

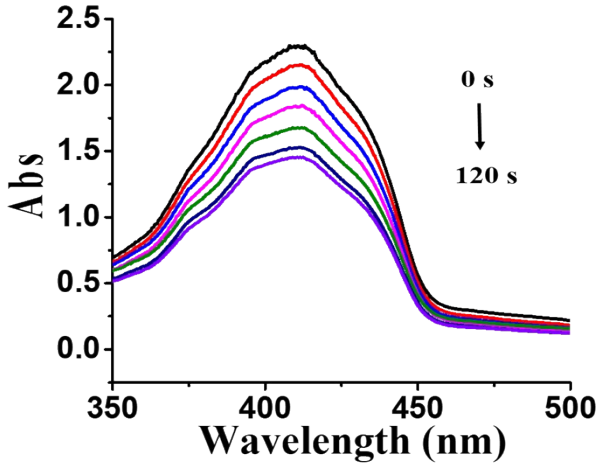

Figure S4. UV absorbance changes of ICG@PDA+ H_2O_2 under the irradiation of 808 laser.

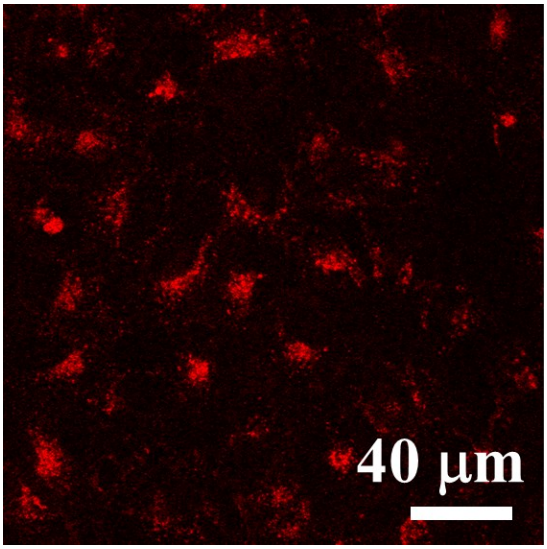

Figure S5. Confocal image of MCF-7 cells treated with Texas Red labelled Pt-ICG@PDA nanoparticles for 6 h.

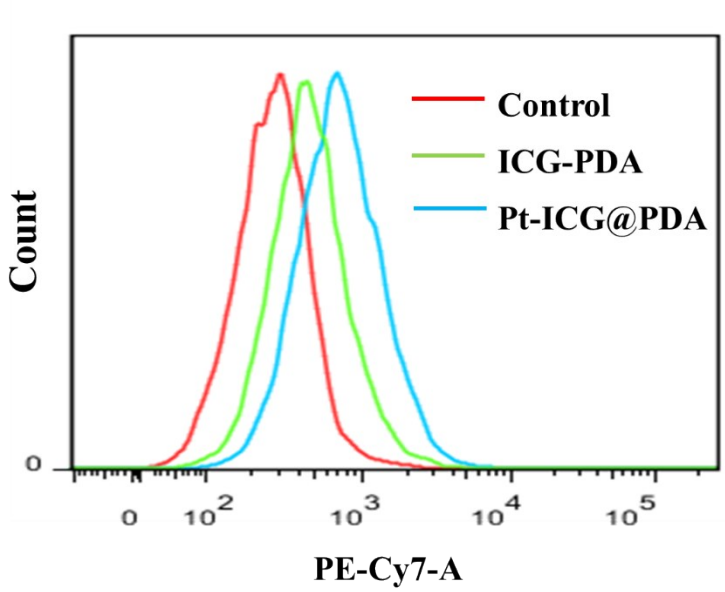

Figure S6. The flow cytometry analysis of MCF-7 cells incubated with ICG@PDA and Pt-ICG@PDA nanoparticles.

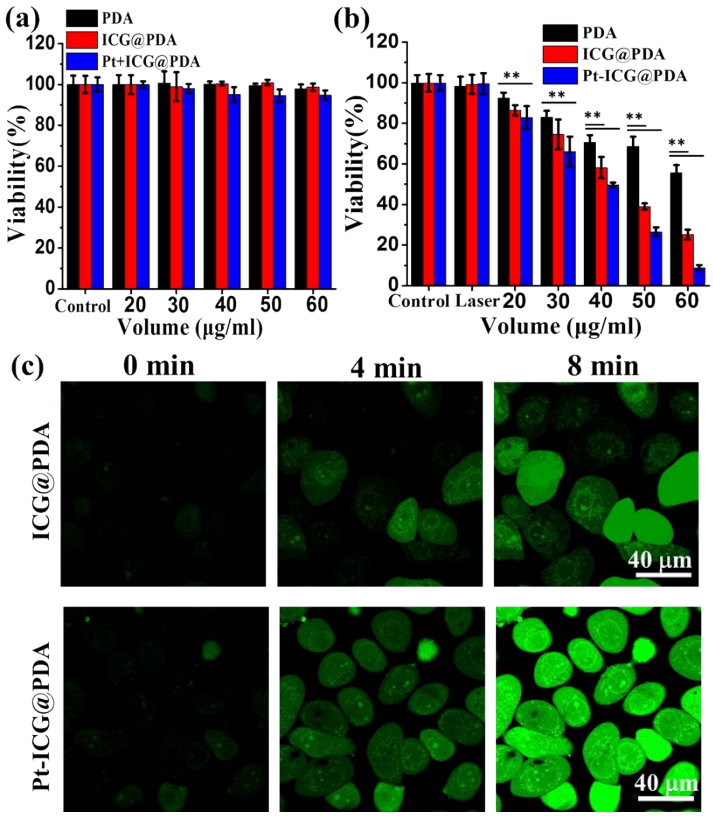

Figure S7. Cytotoxicity detections with MCF-7 cells. (a) Dark cytotoxicity of MCF cells treated with PDA, ICG@PDA, Pt-ICG@PDA without irradiation. (b) Cytotoxicity of MCF-7 cells treated with PDA, ICG@PDA, Pt-ICG@PDA under the irradiation of 808 laser (4 W/cm^2) for 2 min. Data was presented by mean ± SD (n = 4), $^{**}P < 0.01$. (c) ROS detection of cells (incubated with ICG@PDA and Pt-ICG@PDA) irradiated by 808 nm laser for 4 min and 8 min. All above samples were modified with folate-liposome.

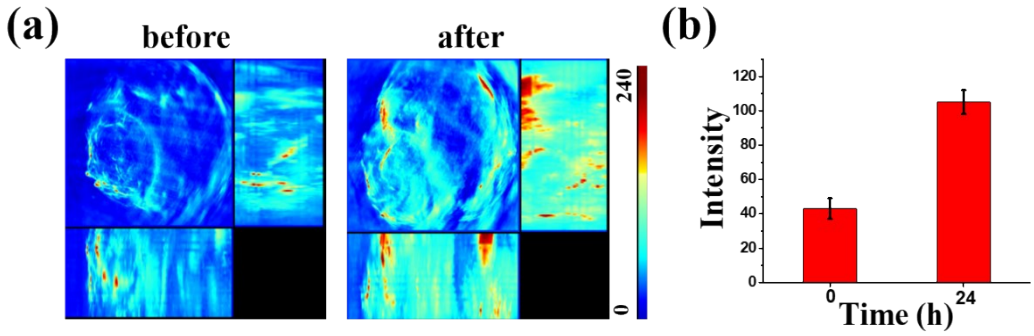

Figure S8. PA study *in vivo* before and after the tail intravenous injection. (a) PA images before and after the injection of Pt-ICG@PDA for 24 h. (b) The intensity values of Figure S8a.

References:

1. Y. Wang, J. Ren, K. Deng, L. Gui and Y. Tang, *Chem. Mater.*, 2000, **12**, 1622-1627.
2. H. Li, Y. Zhao, Y. Jia, C. Qu and J. Li, *Chem Commun*, 2019, **55**, 15057-15060.