Supporting Information

Two-dimensional Intermetallic PtBi/Pt Core/Shell Nanoplates Overcome Tumor Hypoxia for Enhanced Cancer Therapy

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Supplementary Experiment Section

Materials

Platinum acetylacetonate ($Pt(acac)_2$) was purchased from J&K Reagent Co. Ltd. Bismuth neodecanoate ($C_{30}H_{57}BiO_6$) was purchased from Sigma. Acetone, diethylene glycol (DEG) and polyvinyl pyrrolidone (PVP) (molecular weight (MW) = 24000) were purchased from Aladdin Reagent Co. Ltd. Methoxy-PEG-sulphydryl (mPEG-SH) was purchased from Shanghai Tuo Yang Biotechnology Co. Ltd. All chemicals were used as received without further purification.

Synthesis of PtBi/Pt nanoplates

17 mL diethylene glycol (DEG) was firstly heated at 60 ^oC in a vacuum until the impurities are completely removed. $Pt(acac)_2$ and $C_{30}H_{57}BiO_6$, (the mole ratio of Pt and Bi precursor was 2:1) were pre-dissolved in 3 mL DEG and then injected into a flask. The mixture was heated to 150 ^oC and kept for 30 min. After the system cooled to room temperature, black products were obtained and then washed with mixtures of ethanol and acetone. The final product was kept in a 4 ^oC refrigerator. For preparation of PEGylated PtBi NPs, mPEG-SH and PtBi NPs (mass ratio was 4:1) were firstly mixed in aqueous solution, and homogenized by ultrasonic treatment for 30 min, followed by stirring overnight at room temperature. The final product was purified by ultrafiltration to remove the excess mPEG-SH.

Instrument and characterization

UV-Vis spectra were monitored by a UV spectrophotometer (UV-2450, Shimadzu Corporation, Japan). Transmission electron microscopy (TEM) images were recorded on a transmission electronic microscope (JEM-2100Plus, JEOL Ltd., Japan). High-resolution transmission electron microscopy (HRTEM) images were recorded on an FEI transmission electronic microscope (Tecnai G2 F20 S-TWIN, FEI). The average hydrodynamic diameter and zeta potential were determined by dynamic light scattering (Zetasizer Nano ZS90, Malvern Panalytical Ltd). The concentration of the PtBi-PEG aqueous solution was determined by inductively coupled plasma mass

spectrometry (ICP-MS 8900, Agilent Technologies, Inc., USA). A dissolved oxygen meter (HI9146, HANNA instruments, Korea) was used to measure the O_2 concentration. An IR thermal image camera (FLIR E60, FLIR Systems, Inc., USA) was used to record the changes of temperature in 1 min intervals. The radiotherapy experiments were performed by biological X-ray irradiator (X-RAD 225, United Well, USA).

In vitro detection of O_2 concentration

To verify the catalytic activity, different concentrations of PtBi-PEG (0, 1, 2, 5, 10 ppm) with H_2O_2 (10 mM) were added to the centrifugal tubes (4 mL) and stirred and the O_2 concentration was monitored. Further, to verify that irradiation with a 808 nm laser could improve the O_2-producing ability, different concentrations of PtBi-PEG (0, 1, 2, 5, 10 ppm) with H_2O_2 (10 mM) were added to the centrifugal tubes (4 mL) and irradiated by 808 nm laser (1.0 W/cm^2) to monitor temperature changes and generation of O_2 in real time. Finally, in order to simulate weak acidity in the tumor microenvironment, O_2 production at different pHs was detected. PtBi-PEG (10 ppm) with H_2O_2 (10 mM) at different pHs (5.4, 6.8 and 7.4) was added to centrifugal tubes (4 mL) to monitor generation of O_2 in real time.

Cell Cultures

4T1 cells were cultured in Dulbecco's Modified Eagle's medium (DMEM) medium, L02 cells were cultured in RPMI-1640 medium. These two media included 1% penicillin-streptomycin (PS) and 10% fetal bovine serum (FBS).

Intracellular detection of O_2 generation

Intracellular O_2 generation was detected by ROS fluorescence probe-2', 7'-dichlorofluorescein diacetate (DCFH-DA). 4T1 cells were firstly seeded in a Confocal Dish (9.0×10^5 cells/well) for 24 h. Then, PtBi-PEG (10 ppm) was added and co-cultured for another 5 h. After removal of the the previous medium and washed for three times, 1 mL fresh medium containing H_2O_2 (10 mM) was co-cultured for another 30 min and washed for three times. Next, 1 mL fresh medium containing DAPI (1 ppm) and DCFH-

DA (10 μM) was incubated for 15 min and washed with PBS for three times. DPBS was added and fluorescence confocal imaging was performed.

In vitro photothermal studies

PtBi-PEG (100 ppm, 100 μL) was irradiated with a 808 nm laser with various power densities (0.4, 0.8, 1.0 and 1.2 W/cm^2) in eppendorf tubes to monitor temperature changes in real time. Next, different concentrations of PtBi-PEG (0, 10, 20, 50 and 100 ppm, 100 μL) were irradiated in a tube with a 808 nm laser (1.2 W/cm^2) to monitor the temperature changes in real time.

In vitro PA imaging studies

200 μL PtBi-PEG with concentrations ranging from 10 to 100 ppm was added to eppendorf tubes. Then, the PA imaging was performed on an inVision 256-TF imaging system with excitation wavelength of 810 nm.

Cell cytotoxicity

4T1 cells and Human normal hepatocyte (L02) cells were seeded in 96-well plates and cultured with 5% of CO_2 at 37 ^oC. PtBi-PEG was added with a series of concentrations (0, 10, 20, 40, 60, 80, 100, 200, 300 and 400 ppm) and co-cultured for 24 h. Standard methyl thiazolyltetrazolium (MTT, Sigma-Aldrich LLC. Shanghai) assay was carried out to assess the cell viability.

Clonogenic assay and γ-H2AX immunoflourescence analysis

4T1 cells were firstly seeded in 6-well plates and cultured for 24 h, and co-cultured with PtBi-PEG (20 ppm) for another 3 h. 4T1 cells cultured with medium only were set as control groups. Then, the 6-well plates were treated with X-ray at different doses, followed by fixing with formaldehyde and staining with Giemsa. For γ-H2AX immunofluorescence analysis, 4T1 cells were seeded in 12-well plates and incubated for 24 h. After co-cultured with DPBS and PtBi-PEG (100 ppm) for 3 h, the cells were irradiated by X-ray (6 Gy). The cells were then stained with primary mouse monoclonal anti-phospho-histone H2AX antibody (dilution 1:1000) overnight at 4 ^oC, and further stained with Cy5-conjugated sheep anti-mouse secondary antibody (dilution 1:1000).

After that, the cell nucleus was stained with DAPI for 5 min at room temperature. The fluorescence imaging was performed using a laser confocal scanning microscope.

Xenograft tumor mouse model

BALB/c mice were obtained from Hunan SJA Laboratory Animal Co., Ltd. All animal procedures were performed in accordance with the Guidelines for Care and Use of Laboratory Animals of Hunan University and experiments were approved by the Animal Ethics Committee of College of Biology (Hunan University). For tumor inoculation, 4T1 tumor cells were firstly digested and washed for three times, then dispersed in DPBS. Each mouse was injected with 50 μL DPBS containing 5×10^6 cells into the right rear flank.

In vivo multimodal imaging

Tumor-bearing mice were intravenously injected with PtBi-PEG solution (2.0 mg/mL, 200 μL) and the imaging was performed when the mice were anesthetized at different time points. X-ray imaging was obtained on a Xenogen IVIS Lumina XR system. PA imaging was performed by a multi-spectral optoacoustic tomography (MSOT) system (inVision 256-TF, iThera Medical GmbH, Germany) at 810 nm for PA imaging. IR imaging was carried out with an IR thermal imaging camera while the tumor sites of mice were irradiated by 808 nm laser (0.2 W/cm^2, 10 min).

HIF-1α staining

To study the O_2 production capacity of the PtBi-PEG in tumor sites, tumor-bearing mice were intravenously injected with PtBi-PEG solution (2.0 mg/mL, 200 μL). The mice were sacrificed at different time (0, 6, 12, 24 and 48 h) post injection. The tumors were collected for HIF-1α staining to assess the levels of hypoxia in the tumor, which was conducted by Well Biological Science Co. Ltd. To investigate the effect of laser irradiation on the catalytic ability of PtBi-PEG, tumor-bearing mice were intravenously injected with PtBi-PEG solution (2.0 mg/mL, 200 μL), treated as follows: DPBS, PtBi-PEG, PtBi-PEG + Laser (0.2 W/cm^2, 10 min, 24 h post injection). The tumors were collected and sliced for HIF-1α staining by Servicebio Co. Ltd.

In vivo biodistribution and blood circulation

To study the biodistribution of the PtBi-PEG, tumor-bearing mice, were intravenously injected with PtBi-PEG (2.0 mg/mL, 200 μL), and the mice (n = 4) were sacrificed at different time (1, 12, 24, 48 h and 30 d) post injection. Major organs and tissues were weighed and digested. The Pt and Bi content in organs or tissues was determined by ICP-MS. To study the blood circulation, PtBi-PEG (2.0 mg/mL, 200 μL) was intravenously injected and 10 μL of blood was collected from the tail vein of each mouse (n = 4) at the indicated time points and the content of Pt and Bi in blood was determined by ICP-MS.

In vivo PTT-RT synergistic therapy

Tumor- bearing mice were treated with PBS, PtBi-PEG only, X-ray only, Laser + X-ray, PtBi-PEG + Laser, PtBi-PEG + X-ray, and PtBi-PEG + Laser + X-ray (n = 7). The mice received 808 nm laser irradiation (0.2 W/cm^2,10 min) at 44 h and X-ray irradiation (6 Gy) at 48 h post i.v. injection of PtBi-PEG (2.0 mg/mL, 200 μL). The calculation formula of tumor size was as follows: tumor volume = (length × $width^2$)/2. After 18 days of treatment, the main organs in the control group and the PtBi-PEG + Laser + X-ray irradiation group were collected for H&E staining. For blood biochemical and hematological analyses, healthy mice were intravenously injected with PtBi-PEG (2.5 mg/mL, 200 μL) for 30 day. For comparison, the untreated mice served as a control group. After 30 days, blood samples were collected from two groups using standard protocols and sent to Servicebio Co. Ltd for blood analysis.

Statistical analysis

The results are shown as the mean ± standard deviation of the mean. Data analysis in the work was carried out using the Student's t-test, where * $p<0.05$, ** $p<0.01$, *** $p<0.001$. $p<0.05$ was considered statistically significant.

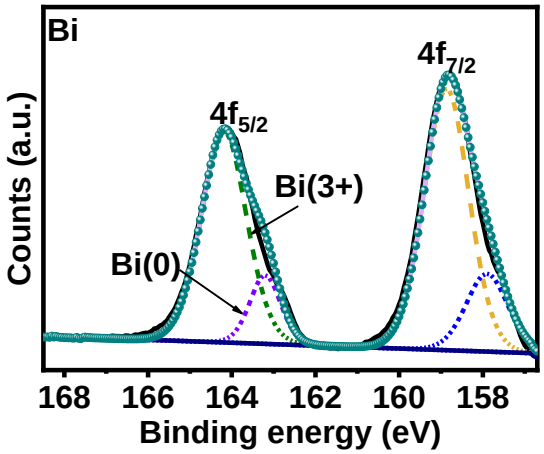

Figure S1. XPS spectra in the region of Bi 4f peak.

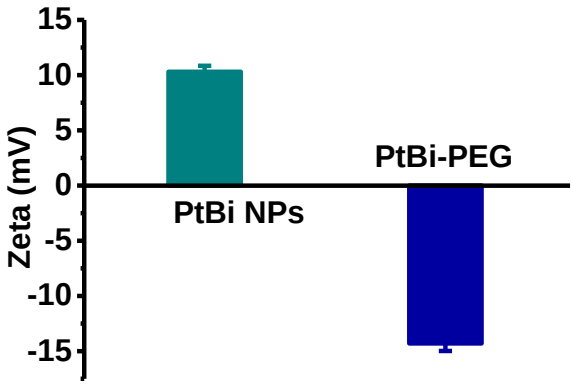

Figure S2. Zeta potentials of PtBi NPs and PtBi-PEG.

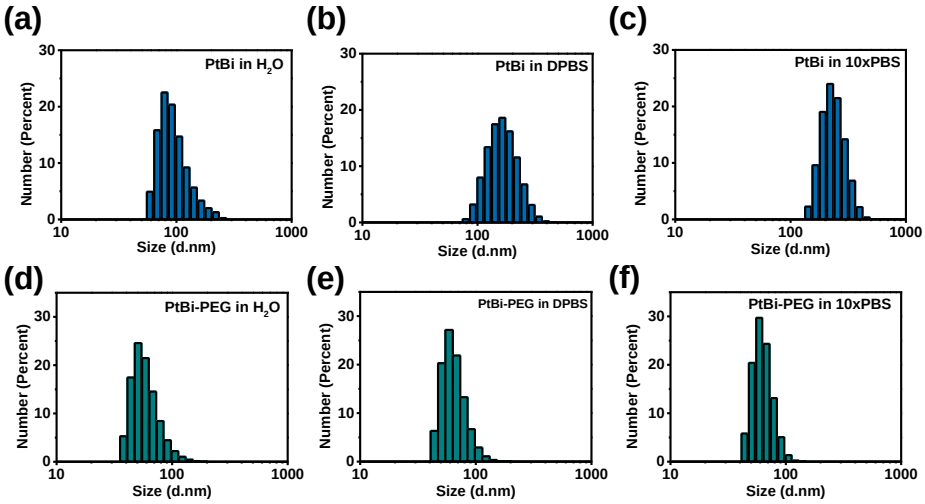

Figure S3. Hydrodynamic size distribution of PtBi NPs and PtBi-PEG in H_2O, DPBS, and 10 × PBS, respectively.

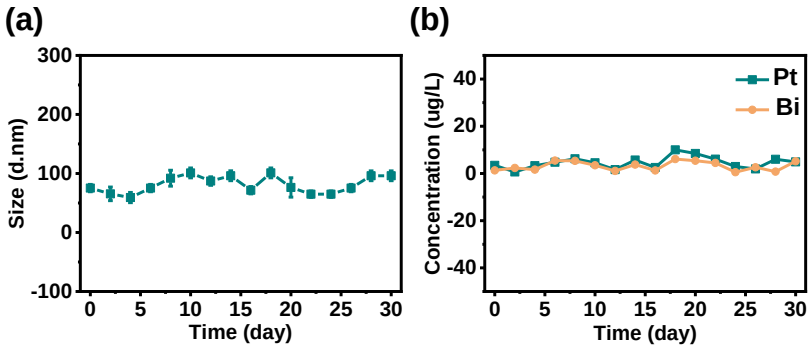

Figure S4. (a) Hydrodynamic size of PtBi-PEG in H_2O over a 30-day interval; (b) Concentrations of $Pt^{2+}$and Bi^{3+} in PtBi-PEG over a 30-day interval by ICP-MS.

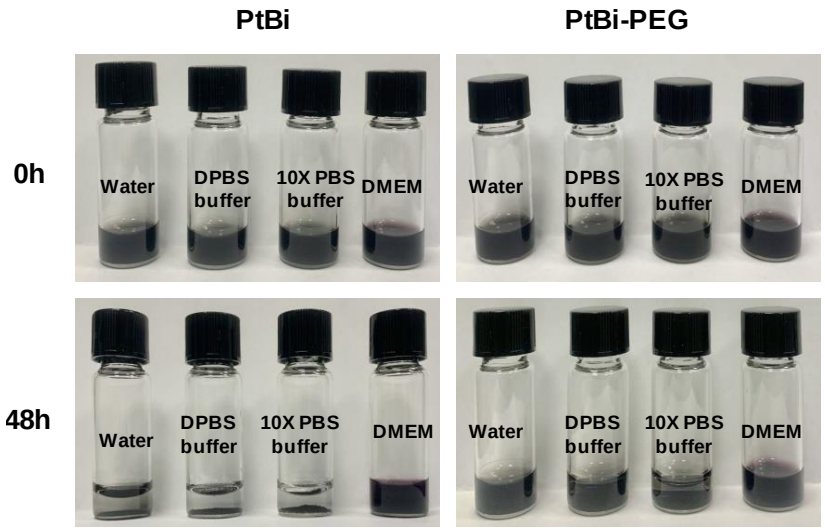

Figure S5. Digital photos of dispersion of PtBi NPS and PtBi-PEG in different solutions.

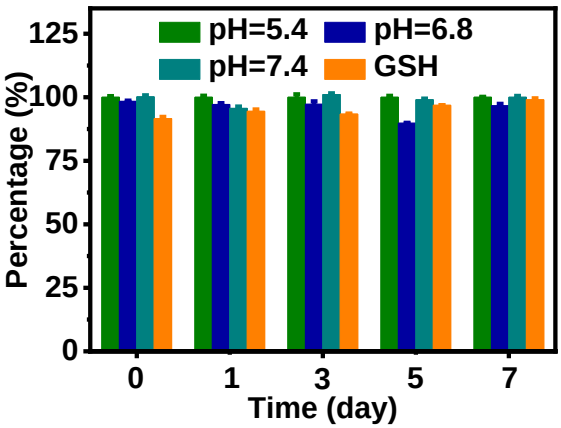

Figure S6. Stability test on the absorption of PtBi-PEG at 808 nm with different treatments at 0, 1, 3, 5 and 7 days.

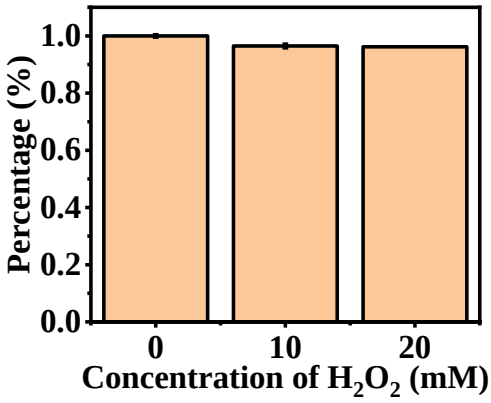

Figure S7. Stability test on the optical absorption of PtBi-PEG at 808 nm with H_2O_2 treatment.

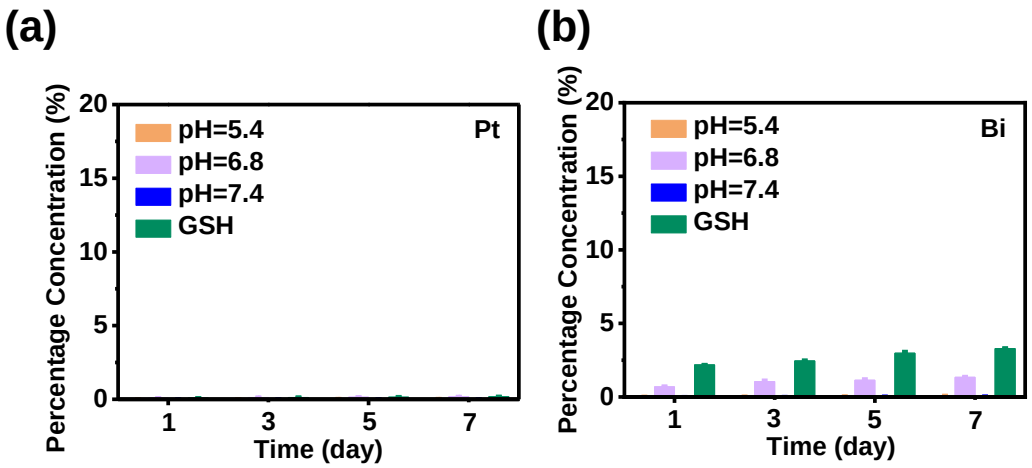

Figure S8. Concentrations of Pt^{2+} (a) and Bi^{3+} (b) in PtBi-PEG with different treatments at 1, 3, 5 and 7 days by ICP-MS.

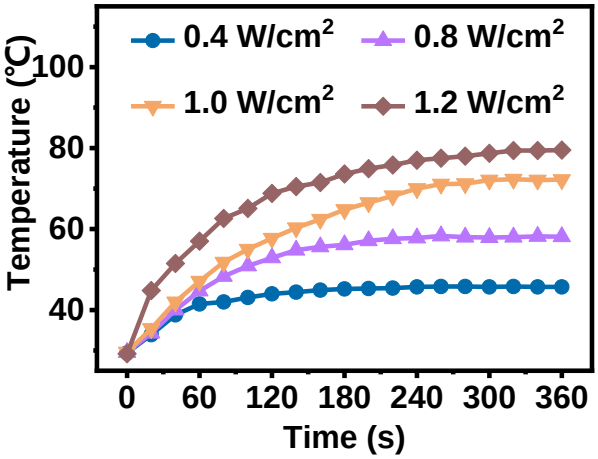

Figure S9. Temperature elevation curves of PtBi-PEG upon irradiation of 808 nm laser with various power densities (0.4, 0.8, 1.0, 1.2 W/cm^2, 100 μL).

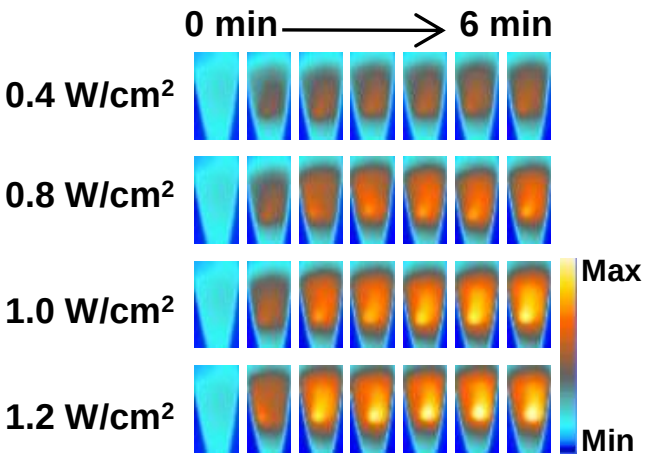

Figure S10. IR thermal images of PtBi-PEG upon irradiation with an 808 nm laser at different power densities (0.4, 0.8, 1.0 and 1.2 W/cm^2, 6 min) in Eppendorf tubes.

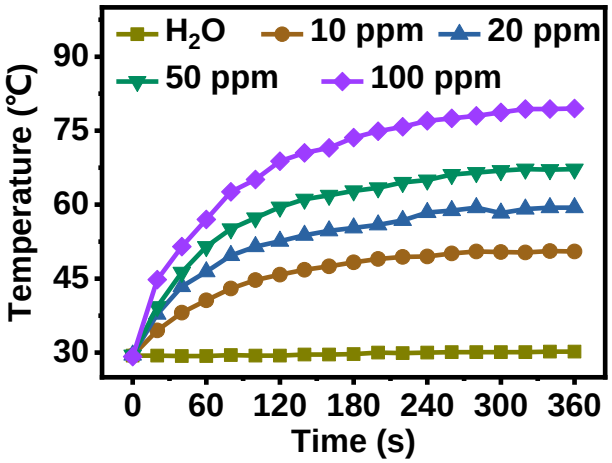

Figure S11. Temperature elevation curves of PtBi-PEG with various concentrations (0, 10, 20, 50 and 100 ppm, 100 μL) under 808 nm laser (1.2 W/cm^2, 6 min) irradiation in Eppendorf tubes.

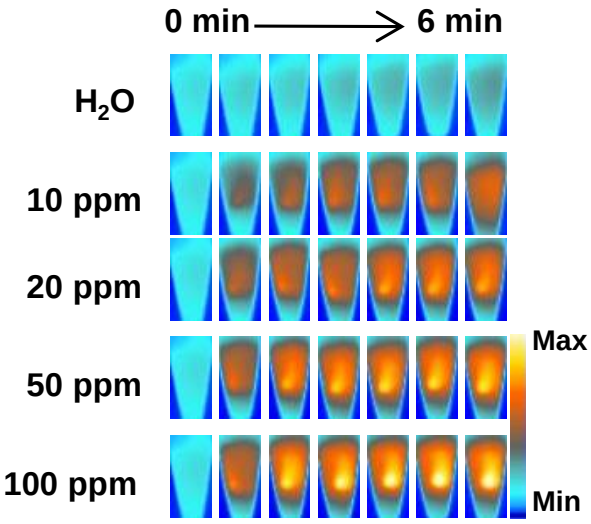

Figure S12. IR thermal images of PtBi-PEG with various concentrations (0, 10, 20, 50 and 100 ppm, 100 μL) under 808 nm laser (1.2 W/cm^2, 6 min) irradiation in Eppendorf tubes.

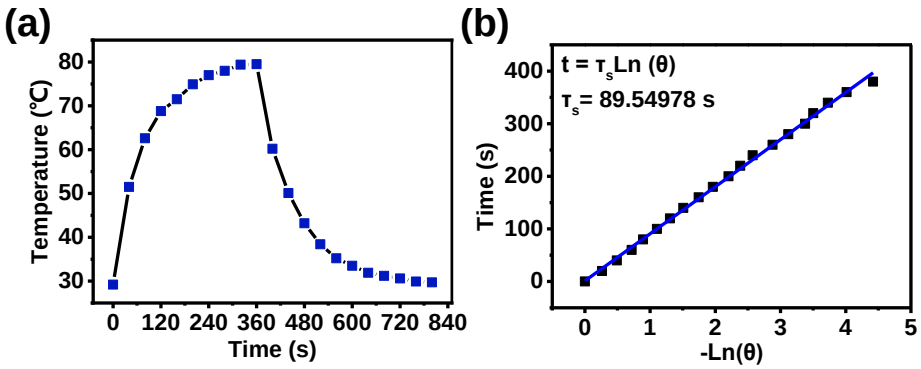

Figure S13. (a) Heating and cooling curve (100 ppm, 1.2 W/cm^2, 100 μL)；(b) Linear time data versus -Ln(θ) of PtBi-PEG under 808nm laser irradiation for 6 min and spontaneous cooling (100 ppm, 1.2 W/cm^2, 100 μL).

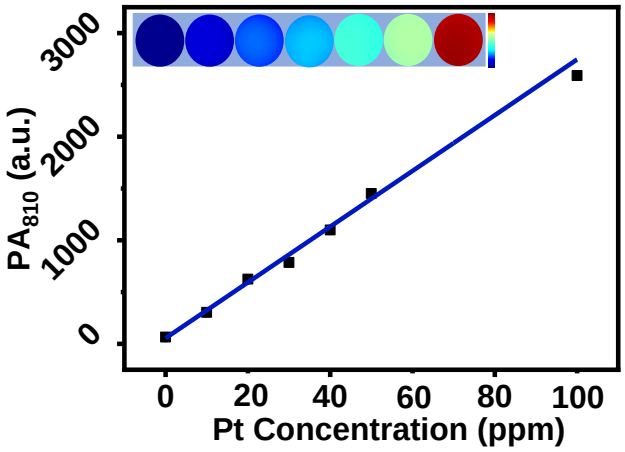

Figure S14. PA signal intensities of PtBi-PEG with different concentrations (810 nm excitation, 200 μL).

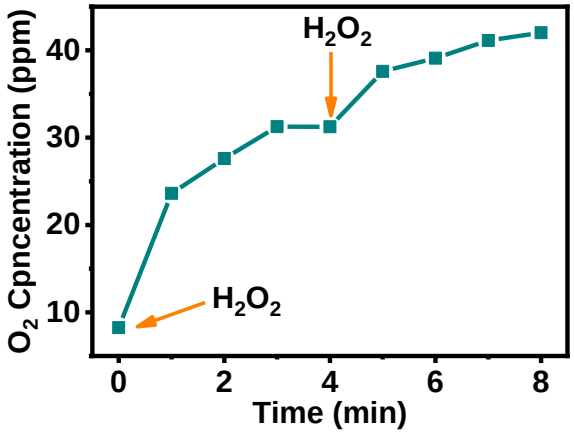

Figure S15. The O_2 concentration of the solution treated with PtBi-PEG in the case of repeated addition of H_2O_2.

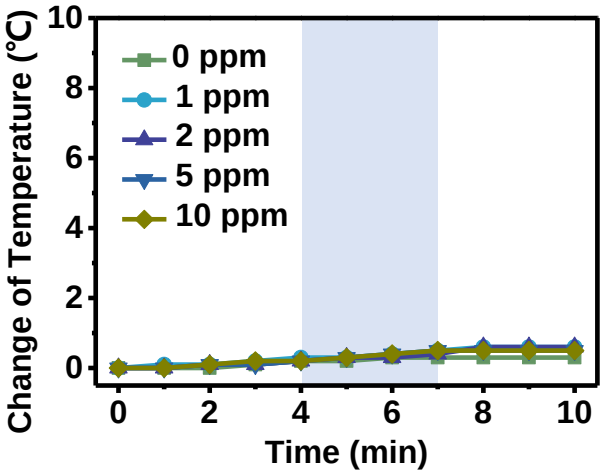

Figure S16. The temperature changes of H_2O_2 (10 mM) incubated at different concentrations of PtBi-PEG under 808 nm laser irradiation from the 4th min to 7th min in vitro.

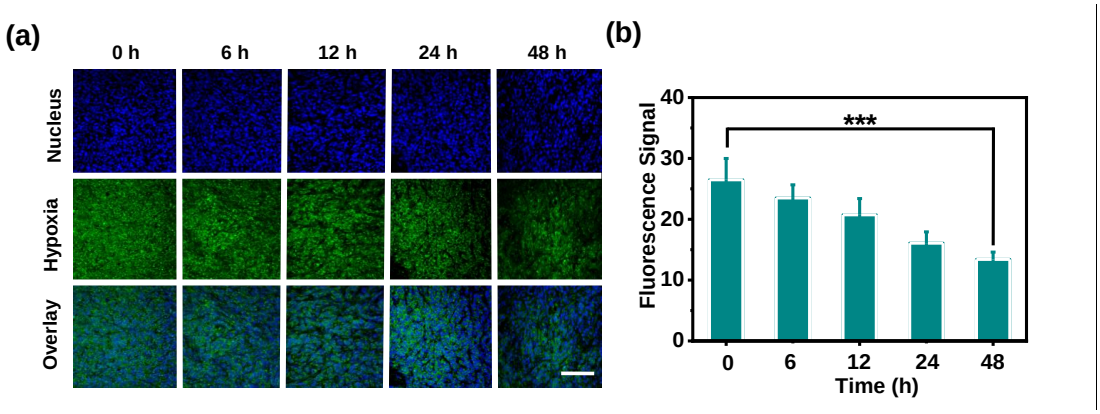

Figure S17. (a) HIF-1α staining of 4T1 tumor after injection with PtBi-PEG (200 μL, 2.0 mg/mL) intravenously for 0, 6, 12, 24 and 48 h (Blue: cell nucleus; Green: HIF-1α, scale bar, 100 μm); (b) HIF-1α Fluorescence intensity statistics of different groups revealed in (a).

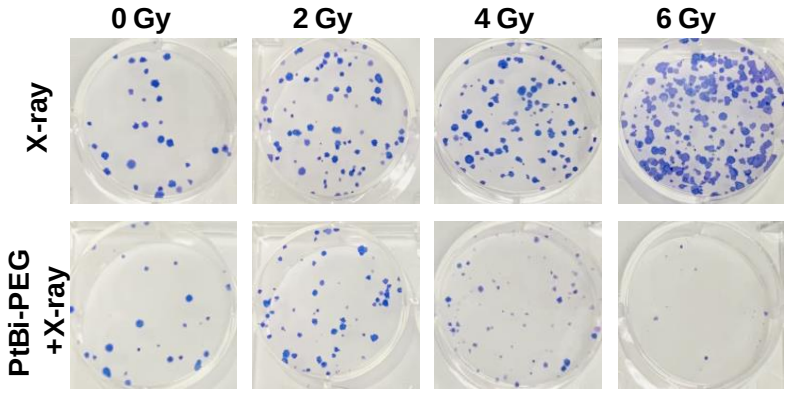

Figure S18. Optical photographs of clonogenic survival assay.

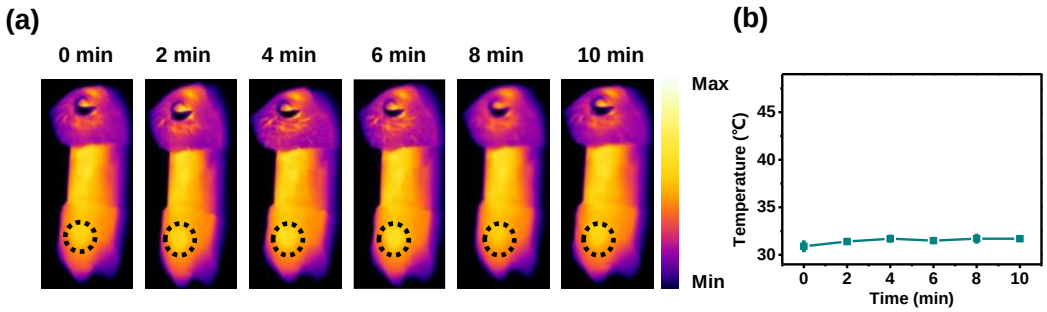

Figure S19. IR thermal images of tumor sites without PtBi-PEG injection upon laser irradiation (808 nm, 0.2 W/cm^2, 10 min).

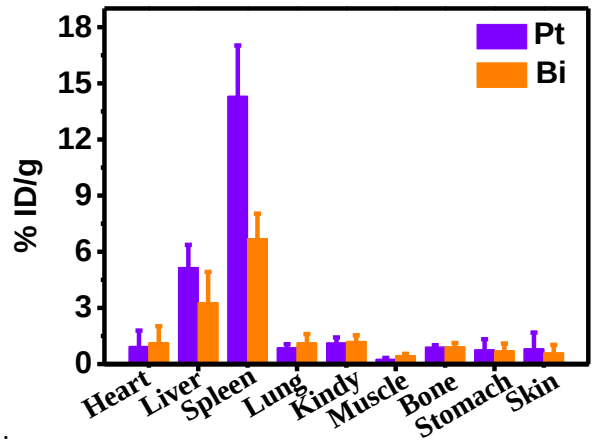

Figure S20. Biodistribution of PtBi-PEG in major organs at 30 days i. v. post-injection of PtBi-PEG (200 μL 2.0 mg/mL).

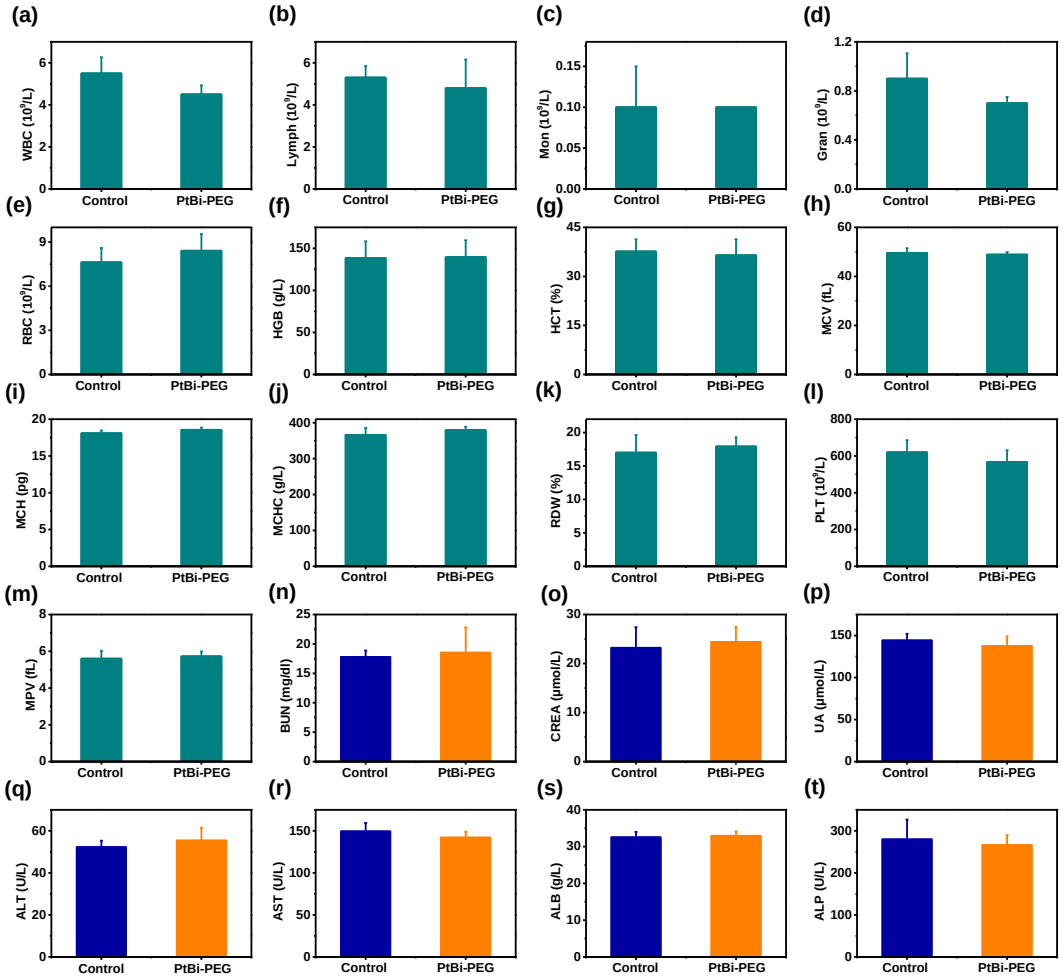

Figure 21. Biochemical and hematological analysis of blood. Indicators to be checked include (a) white blood cell (WBC); (b) lymphocyte (Lymph); (c) monocytes (Mon); (d) neutrophil (Gran); (e) red blood cell (RBC); (f) hemoglobin (HGB); (g) hematocrit (HCT); (h) erythrocyte mean corpuscular volume (MCV); (i) mean corpuscular hemoglobin (MCH); (j) mean corpuscular hemoglobin concentration (MCHC); (k) red cell volume distribution width (RDW); (l) platelet（PLT）； (m) mean platelet volume (MPV); (n) blood urea nitrogen (BUN); (o) creatinine (CREA); (p) uric acid (UA); (q) alanine aminotransferase (ALT); (r) aspartate aminotransferase (AST); (s) albumin (ALB); (t) alkaline phosphatase (ALP).