Supporting Information

Multifunctional Bismuth Nanoparticles as Theranostic Agent for PA/CT Imaging and NIR Laser Driven Photothermal Therapy

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EXPERIMENTAL SECTION

Materials. All reagents were analytical grade and used as received without any further purification. Bismuth (III) acetate, 5,5′,6,6′-tetrachloro-1,1′,3,3′-tetraethyl-imidacarbocyanine iodide (JC-1), 3-(4,5-dimethylthialzol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), calcein acetoxymethyl ester (calcein-AM), 4′,6-diamidino-2-phenylindoles (DAPI) and propidium iodide (PI) were obtained from Sigma-Aldrich (China). Fluorescence-labeled 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine-N-(7-nitro-2-1,3-benzoxadiazol-4-yl) (NBD PE) was obtained from Invitrogen (China). Ethidium bromide (EB) was purchased from Macklin (China). Oleic acid, 1-dodecanethiol and trioctylphosphine (TOP) were bought from Aladdin (China). 1,2-Dilauroyl-sn-glycero-3-phosphocholine (DLPC) was purchased from Avanti Polar Lipids (USA).

Characterization. Transmission electron microscopy (TEM) images were captured using a FEI Tecnai G2 F30 microscope under an acceleration voltage of 200 kV and a JEM-1400 microscope under an acceleration voltage of 100 kV. The thermogravimetric analysis (TGA, Rigaku, TG8101D) was performed from room temperature to 800°C with a heating rate of 10°C/min in an air atmosphere. Fourier transform infrared (FT-IR) spectra were acquired by AVATAR360 spectrometer. Ultraviolet-visible-near-infrared (UV-vis-NIR) spectra were recorded by a U-4100 spectrophotometer (Hitachi, Japan). X-ray photoelectron spectroscopy (XPS) was conducted on the Thermo Fisher Scientific ESCALAB 250Xi. The crystal structure of the sample was characterized with X'PERT PRO MPD X-ray diffractometer equipped with Cu*K*α radiation. The size distribution of the samples was carried out by dynamic light scattering (DLS) using a Zeta potential analyzer (Zeta PALS BI-90 Plus, Brookhaven Instruments).

Synthesis of Bi NPs. Monodispersed Bi NPs were prepared according to the reported literature with modifications.[1] Briefly, bismuth (III) acetate (1 mmol) was dissolved in the mixed

solution of oleic acid (5 mL) and 1-dodecanethiol (5 mL). The mixture was heated to 40°C for 30 min under vacuum. After that, the flask was filled with nitrogen, and further heated to 100°C to obtain a transparent yellow solution. While holding the final temperature, another freshly prepared TOP (5 mL) solution including 1-dodecanethiol (500 μL) were rapidly injected into the flask to immediately generate a black solution, which indicated the formation of the monodispersed Bi NPs, then the reaction was maintained for 30 s. The black solution was immediately cooled to room temperature in the water bath. The dark product was washed with acetone and chloroform, respectively, and the final product was stored in chloroform for further use.

Formation of DLPC Vesicles. The DLPC vesicles were fabricated using the extrusion method.[2] A chloroform solution containing DLPC (5 mg) was dried by a vacuum desiccator for 3 h to obtain a dry lipid film on a bottle wall, and subsequently vortexed after adding deionized water (1 mL). The cloudy solution was extruded using a mini-extruder (Avanti Polar lipids, Inc. USA) through a polycarbonate membrane with a diameter of 100 nm for 21 times. Finally, the DLPC vesicle solution (5 mg/mL) was prepared and stored in 4°C for further use.

Synthesis of Bi@DLPC NPs. Bi@DLPC NPs (i.e. 500 μg/mL) were readily performed by the ultrasonic method. Briefly, the mixture of the as-prepared Bi NPs (1 mg) and DLPC vesicles (120 μL, 5 mg/mL) were dispersed into deionized water (total volume 2 mL) under ultrasonic treatment for 10 min. Then, the resulting solution was heated up to 60°C for 30 min to eliminate the chloroform. After that, the solution was cooled to room temperature, and sonicated for 5 min. The obtained product was centrifuged to collect the precipitates, washed three times with deionized water. The final product Bi@DLPC NPs were redispersed into deionized water for

further experiments. Bi@DSPE-PEG-2000 and Bi@PVP NPs can be obtained by a similar approach with Bi@DLPC NPs.

Cell Culture. The human breast cancer MDA-MB-231 cells were cultured in DMEM (Dulbecco's Modified Eagle Medium, Corning) high glucose medium, and human normal breast epithelial MCF-10A cells were cultured in RPMI-1640 (Roswell Park Memorial Institute 1640, Corning) medium supplemented with 10% (v/v) fetal bovine serum (FBS, Gibco) and 1% antibiotics (penicillin-streptomycin) (Corning). All cells were cultured in a humidified incubator at 37°C including 5% CO_2.

In Vitro Cell Viability Assay. To investigate the cell viability in vitro, the cell viability in the presence of Bi@DLPC NPs was evaluated using a standard 3-(4,5-dimethylthialzol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. In detail, MDA-MB-231 cells and MCF-10A cells were seeded into a 96-well plate (1×10^4 cells per well) in DMEM containing 10% FBS and 1% antibiotics, and incubated in a humidified 5% CO_2 atmosphere at 37°C for 12 h. Then, DMEM medium was replaced with 200 μL of the fresh cell culture medium containing different concentrations of Bi@DLPC NPs (0, 15.63, 31.25, 62.5, 125, 250, 500 μg/mL) for sequentially incubated for 24 h. MTT solution (20 μL, 5 mg/mL in PBS) was added to each well to incubate for another 4 h at 37°C. After the cell culture medium containing MTT was removed, DMSO (150 μL) was added to each well for 30 min. The optical absorbance was then measured at 490 nm using microplate reader (SynergyTM HT, BioTek Instruments Inc., USA).

Intracellular Distribution by Confocal Laser Scanning Microscopy (CLSM). For intracellular distribution evaluation, MDA-MB-231 cells were seeded into a 35-mm petri dish (2×10^4 cells per dish) and grown for 12 h in supplemented medium at 37°C. The cell culture medium was then replaced by the fresh cell culture medium containing Bi@DLPC NPs with

fluorescent-labeled NBD PE (125 μg/mL) and incubated at 37°C for different time period (0, 1, 3, 6, 12, 24 h). Afterwards the cells were softly washed with PBS and fixed with 4% paraformaldehyde for 10 min at 37°C. After washing three times with PBS, and the nuclei were stained with 4′,6-diamidino-2-phenylindole (DAPI) for another 5 min at 37°C, followed by washing three times with PBS. Finally, MDA-MB-231 cells were observed with confocal laser scanning microscopy (Leica TCS SP5 II).

In Vitro Photothermal Therapy (PTT). The photothermal therapy in vitro was evaluated by counting live/dead cells. MDA-MB-231 cells were seeded into a 35-mm petri dish (3×10^5 cells per dish) and grown for 12 h in 5% CO_2 at 37°C until being nearly 80-90%. The cells culture medium were replaced with 2 mL of the medium containing Bi@DLPC NPs (250 μg/mL) for 6 h. After being washed with PBS for three times, the fresh culture medium was added into petri dishes. The experimental groups were irradiated with NIR laser (880 nm, 1 W/cm^2, diameter of laser beam: 6 mm) for different time (2, 6, and 10 min). After antitumor treatments in vitro, the cell culture medium was removed and rinsed with PBS. The calcein-AM and PI solution in PBS was added to MDA-MB-231 cells to stain the living and dead cells, respectively. After incubated for 20 min at 37°C, the cells were washed with PBS and observed immediately under the fluorescence microscope (Olympus BX53).

Photothermal Performance. To evaluate the photothermal conversion efficiency (η), Bi@DLPC NPs solution was allowed to the laser irradiation at 880 nm in quartz cuvettes until the solution remained a steady temperature. After the laser was turned off, the solution was natural cooled to room temperature. Finally, the photothermal conversion efficiency (η) is calculated according to the previous reported method.

$$\eta = \frac{hS(T_{max}-T_{surr})-Q_{dis}}{I(1-10^{-A_{880}})}$$

In the formula, h is the heat transfer coefficient, S is the surface area of the container, and the value of hS is obtained from **Figure 2e**. T_{max} and T_{Surr} are the equilibrium temperature and ambient temperature of the surroundings, respectively. Q_{dis} is expressed as the heat associated with the light absorbance, I is the incident laser power (1 W/cm^2) and A_{880} means the absorbance of Bi@DLPC NPs at 880 nm. The photothermal conversion efficiency (η) is calculated to be 35% using these parameters.

Elevation of Plasma Membrane Permeability by Photothermal Effect. MDA-MB-231 cells were seeded into a 35-mm petri dish (2×10^4 cells per dish) and incubated for 12 h in DMEM in a humidified 5% CO_2 at 37°C. MDA-MB-231 cells were divided into four groups for control (groups I), NIR laser irradiation only (groups II), Bi@DLPC NPs only (groups III), and Bi@DLPC NPs (250 μg/mL) with NIR laser irradiation (groups IV) in DMEM containing EB (10 μg/mL), and then sequentially cultured for 6 h at 37°C. Afterwards, MDA-MB-231 cells were irradiated with NIR laser for 10 min. Then, the medium was removed, and the cells was washed three times with PBS. The living cells were stained with calcein-AM in PBS (1 μg/mL, 200 μL) for another 20 min at 37°C, followed by washing three times with PBS. Finally, MDA-MB-231 cells were imaged under fluorescent microscope (Olympus BX53).

Mitochondrial Membrane Potential Change. MDA-MB-231 cells were seeded into a 35-mm petri dish (2×10^4 cells per dish) and incubated for 12 h in DMEM in 5% CO_2 at 37°C. MDA-MB-231 cells were assigned to four groups including control (group I), NIR laser irradiation only (group II), Bi@DLPC NPs only (group III), and Bi@DLPC NPs (250 μg/mL) with NIR laser irradiation (group IV) in fresh the medium, and then sequentially cultured for 6 h at 37°C. Afterwards, the medium was removed, then the cells were washed by PBS to remove the excess samples and dead cells. The cells in group II and IV were irradiated under NIR laser

for 10 min. JC-1 (10 μg/mL, 200 μL) solution was used to stain mitochondria according to reported literature.[3] After staining for 20 min, MDA-MB-231 cells were washed to remove extra dye molecules by PBS and fixed with 4% paraformaldehyde for 10 min at 37°C. The nuclei were stained with 4′,6-diamidino-2-phenylindole (DAPI) for another 5 min at 37°C. Finally, MDA-MB-231 cells were observed with confocal laser scanning microscopy (Leica TCS SP5 II).

In Vitro and in Vivo Photoacoustic (PA) Imaging and CT Imaging. The PA images in vitro and in vivo were detected using a PA computed tomography scanner (MOST invision 128). For in vitro PA imaging, Bi@DLPC NPs with various concentrations (0, 125, 250, 500, 1000 μg/mL) in the agar-phantom container were scanned for signal detection at an excitation wavelength of 880 nm. For in vivo PA imaging, Bi@DLPC NPs (2 mg/mL, 100 μL) was intravenously injected into the MDA-MB-231 tumor-bearing mice. PA scanning signals in the tumor sites were recorded before and after injection at different time (0, 1, 3, 6, 12 and 24 h) after anesthetic with isoflurane gas. A computed X-ray tomography scanner (quantum GX micro CT) at 90 kV and 160 μA was employed to accomplish CT imaging. Bi@DLPC NPs with various concentrations (0.313, 0.625, 1.25, 2.5, 5 mg/mL) were added into eppendorf tubes for in vitro CT imaging. For in vivo CT imaging, in brief, the tumor-bearing mice were anesthetized with isoflurane gas, and then Bi@DLPC NPs (4 mg/mL, 100 μL) were intratumorally injected for CT imaging. CT images were collected both before and after injection at different time (0, 0.5, 1, 3, 6, 12 and 24 h).

In Vivo Photothermal Cancer Therapy. The photothermal performance of Bi@DLPC NPs in vivo were investigated in healthy female Balb/c nude mice (4-5 weeks old), which were purchased from Vital River Laboratory Animal Technology Co., Ltd. (Beijing). All animal experiments in vivo were carried out according to the criteria of the National Regulation of

China for Care and Use of Laboratory Animals. For the tumor-bearing animal model, MDA-MB-231 cells (1×10^7 cells in DMEM and Matrigel) were subcutaneously injected into the left front leg of mice by conventional tumor induction method. The tumor-bearing nude mice with approximate volume of 200 mm^3 were randomly separated into five groups (n = 5, each group) including PBS as control (group I), NIR laser irradiation only (group II), Bi@DLPC NPs only (group III), Bi@DLPC NPs with NIR laser irradiation (1 W/cm^2) for 5 (group IV) and 10 min (group V), respectively. The mice were intravenously injected with 100 μL of PBS or Bi@DLPC NPs (1 mg/mL). At 6 h post-injection, tumors of mice were irradiated by NIR laser (1 W/cm^2) for the group II, IV, V. At experimental predetermined time points, thermographic images and temperature changes were monitored using an infrared thermal imaging camera. The tumor volumes and body weights of mice were measured on daily basis and normalized in comparison with their initial values. The tumor volume was defined using the formula: volume = (tumor length) × $(\text{tumor width})^2/2$. Relative tumor volume was defined as V/V_0, similarly relative body weight was defined as W/W_0. V_0 and W_0 are the initial tumor volume and initial mice weight, respectively.

Blood Biochemical Analysis. The blood (20 μL) were collected from mice at 14^{th} day and dissolved in hemodilution solution using for blood biochemical analysis by automatic analyzer (HF-3800).

Histology Analysis. The mice were sacrificed at 14^{th} day to excise the major organs (heart, liver, spleen, lung, and kidney) and tumors for histological analysis, which were fixed in 4% paraformaldehyde solution, and processed routinely in paraffin, followed by sectioning into thin slices. The slices of major organs and tumor tissues were stained with hematoxylin and eosin

(H&E). Finally, optical microscope images were recorded with a fluorescent microscope (Olympus BX53).

In Vivo Biodistribution. To investigate the biodistribution of Bi@DLPC NPs, tumor-bearing mice (n = 3) were intravenously injected with Bi@DLPC NPs (1 mg/mL, 100 μL). At different time post-injection (1, 3, 7, 14 d), the mice were sacrificed, and then the major organs (heart, liver, spleen, lung, and kidney) and tumors were harvested from the mice, and lysed in the digesting solution for 24 h. The contents of Bi in the dissected organs and tumors were determined by inductively coupled plasma optical emission spectrometry (ICP-OES) analysis.

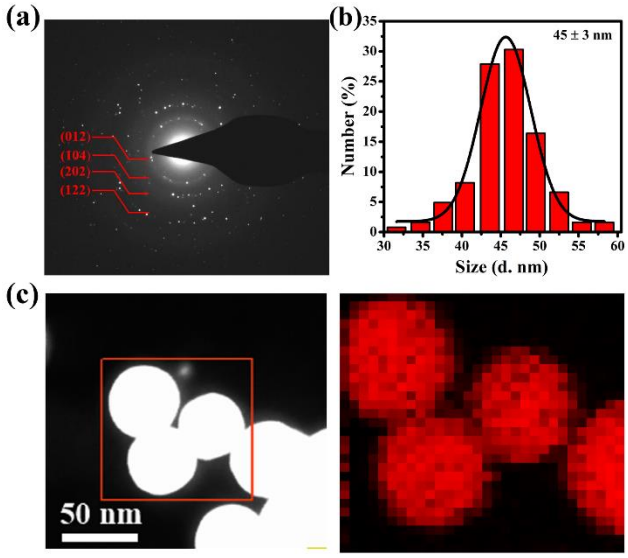

Figure S1. Characterizations of the as-prepared Bi NPs: (a) SAED pattern of Bi NPs. (b) Statistical size distribution analysis of Bi NPs (N = 100). (c) EDS elemental mapping analysis of Bi NPs. The red color in (c) indicates Bi element.

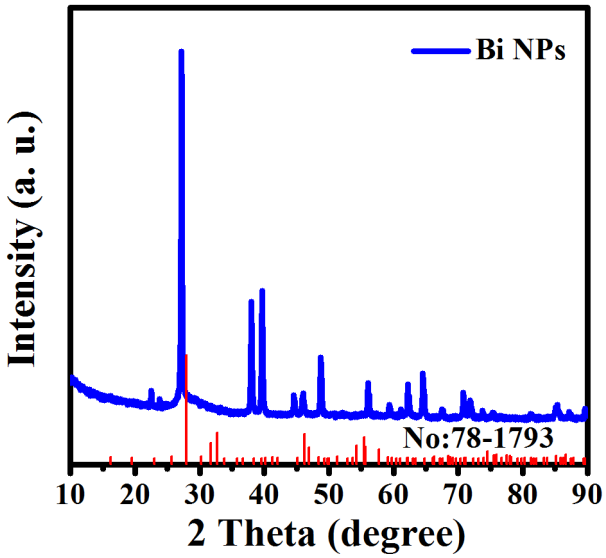

Figure S2. Powder XRD pattern of Bi NPs compared with Bi_2O_3.

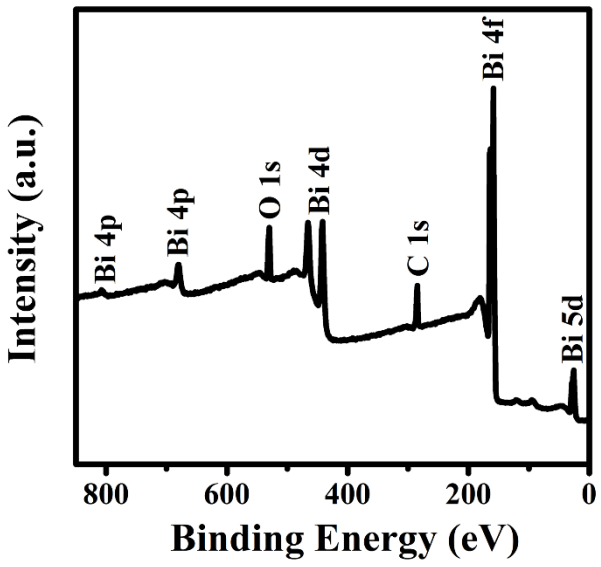

Figure S3. XPS survey of Bi NPs

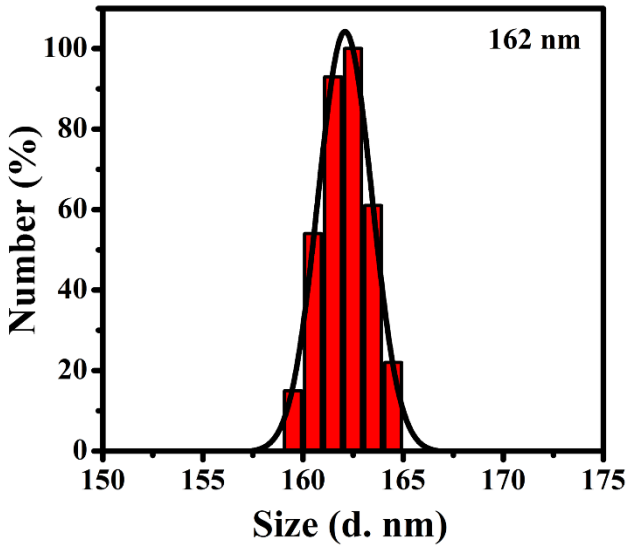

Figure S4. DLS analysis of Bi@DLPC NPs.

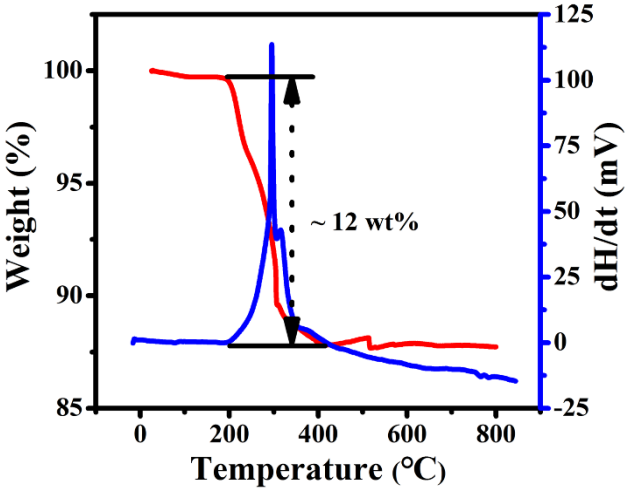

Figure S5. The TGA and DSC curves of Bi@DLPC NPs in air atmosphere.

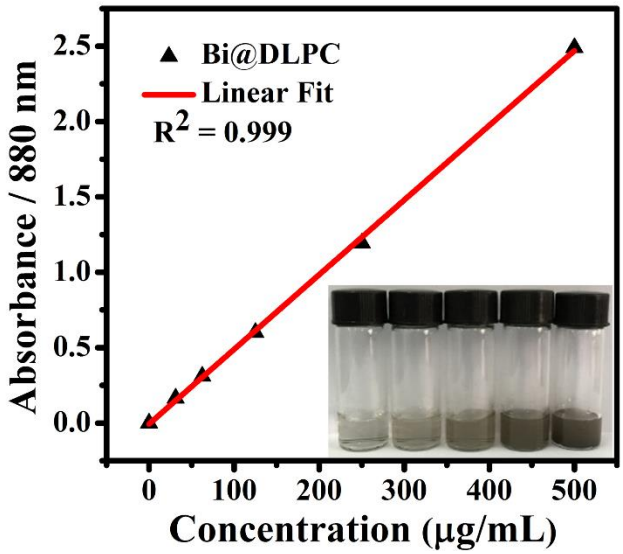

Figure S6. Linear fitting curve of the absorbance versus concentrations (0, 31.25, 62.5, 125, 250, 500 μg/mL) at 880 nm. The inset photographs are the corresponding Bi@DLPC NPs solutions.

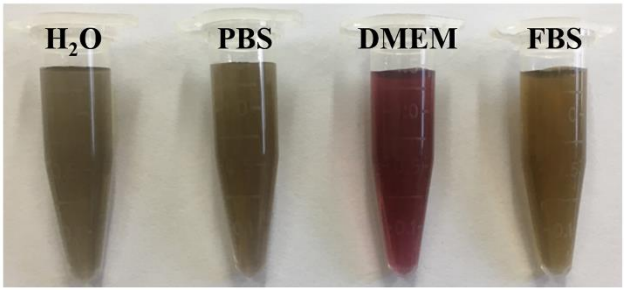

Figure S7. Photographs of Bi@DLPC NPs in deionized water, PBS, DMEM, FBS, respectively.

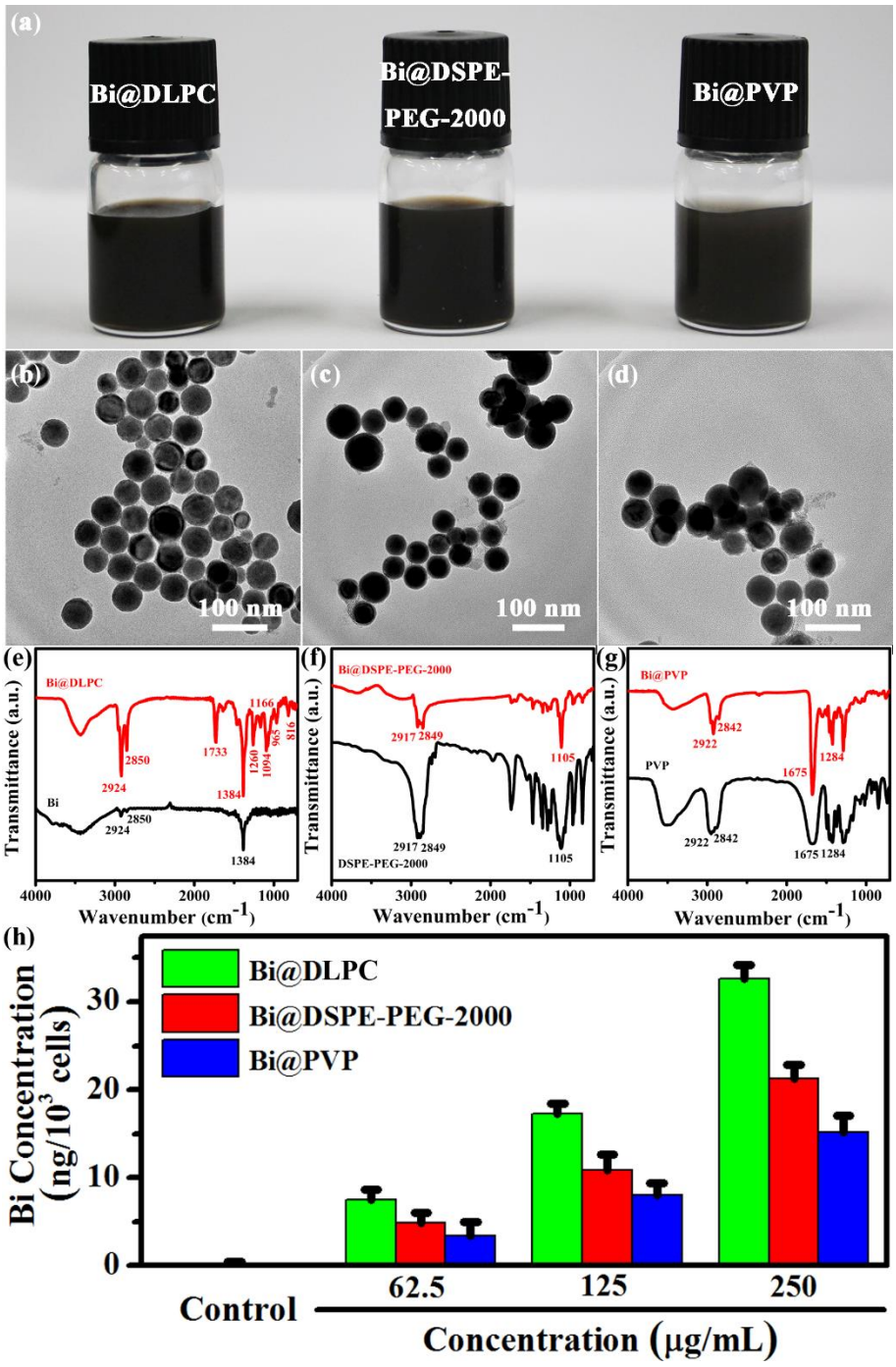

Figure S8. (a) The photographs of surface modified Bi NPs. (b-d) TEM images and (e-g) FT-IR spectra of Bi@DLPC, Bi@DSPE-PEG-2000 and Bi@PVP NPs, respectively. (h) Quantification of intracellular Bi contents of Bi@DLPC, Bi@DSPE-PEG-2000 and Bi@PVP NPs in MDA-MB-231 cells.

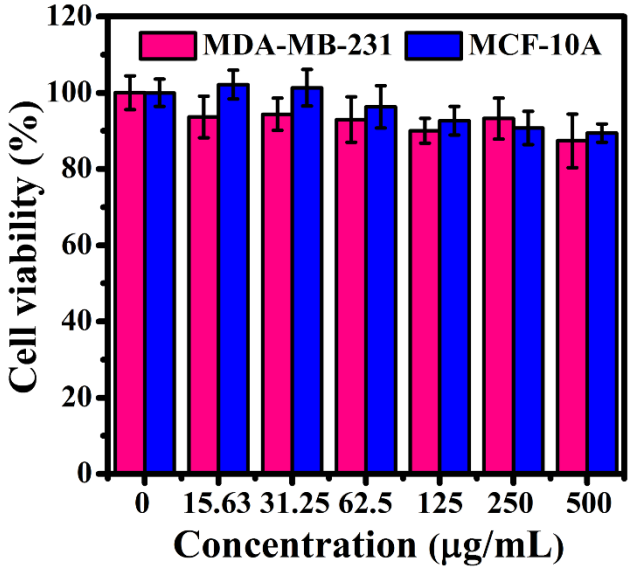

Figure S9. Cell viability assay of MDA-MB-231 and MCF-10A cells treated with various concentrations of Bi@DLPC NPs for 24 h.

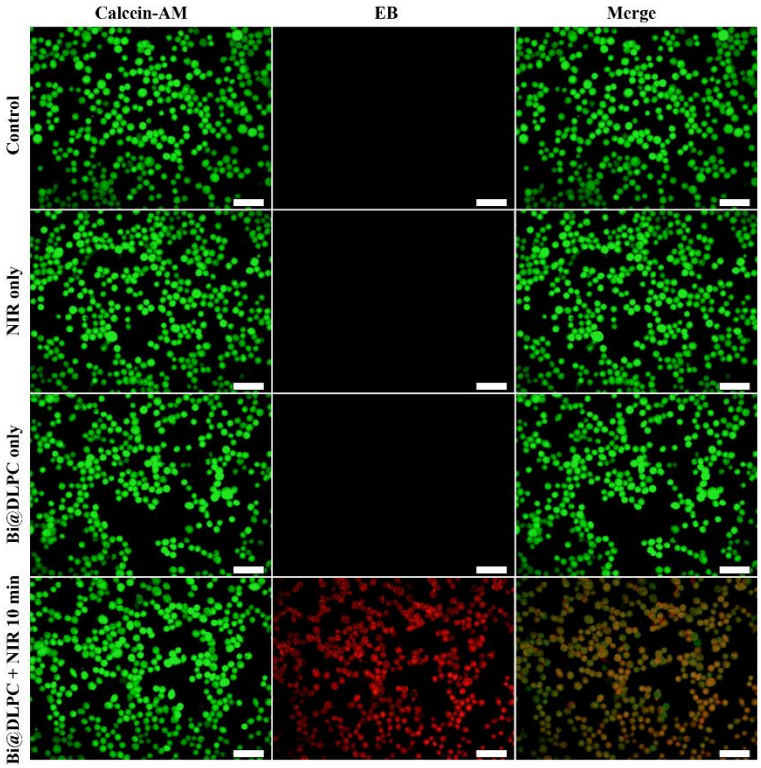

Figure S10. The permeability study of MDA-MB-231 cells with EB/calcein-AM staining after different treatments: control, NIR laser irradiation only, Bi@DLPC NPs only, and Bi@DLPC NPs with NIR laser irradiation (880 nm, 1 W/cm^2) for 10 min (scale bar, 50 μm).

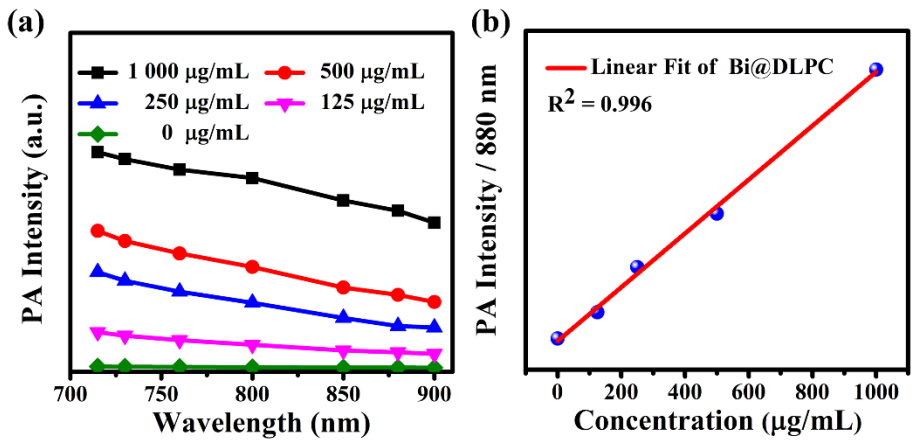

Figure S11. (a) In vitro PA signal intensity of Bi@DLPC NPs at different concentrations. (b) PA signal intensity of Bi@DLPC NPs as a function of concentration at 880 nm.

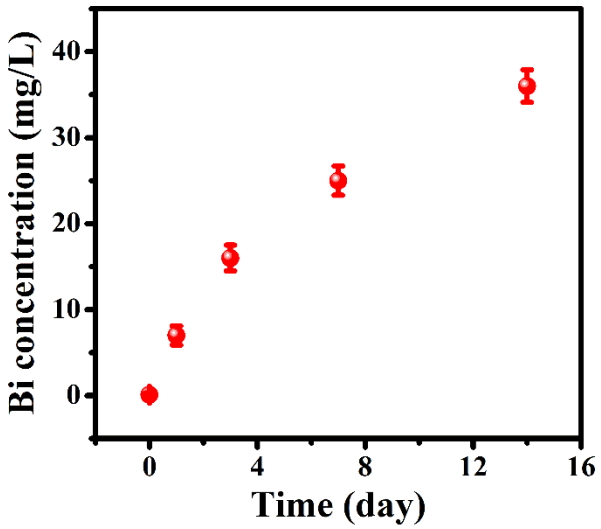

Figure S12. The degradation of Bi@DLPC NPs in simulated liver liquid for different time.

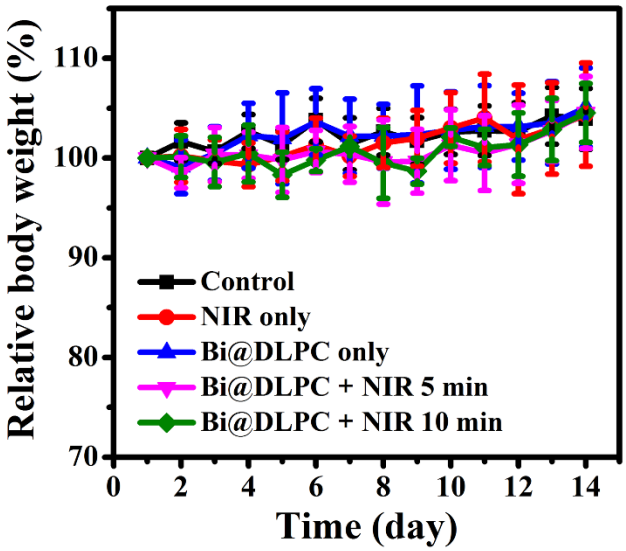

Figure S13. Normalized body weight during the therapeutic period after various treatments (n = 5).

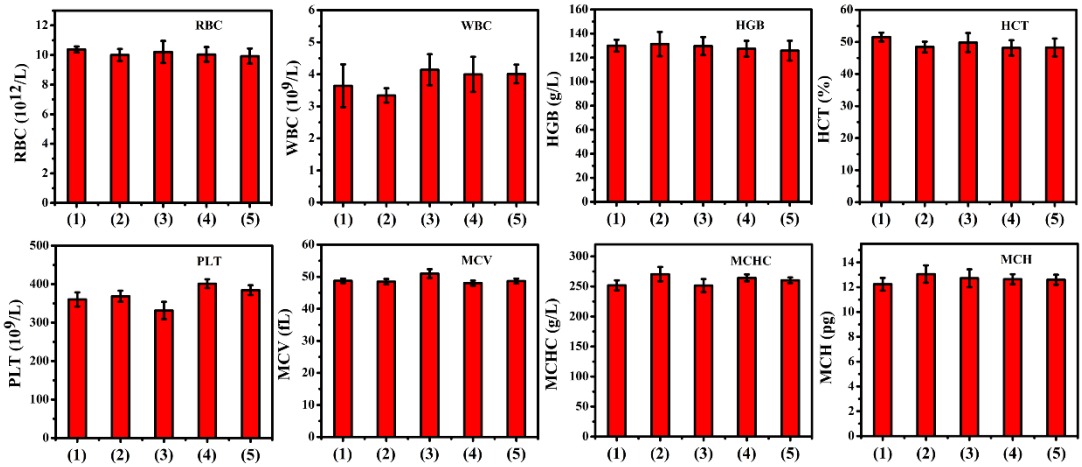

Figure S14. The blood biochemistry analysis of mice at 14th day after different treatments (n = 5): (1) control, (2) NIR laser irradiation only, (3) Bi@DLPC NPs only, (4) Bi@DLPC + NIR for 5 min, (5) Bi@DLPC + NIR for 10 min.

References

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