Near-Infrared Light-Mediated Photodynamic/Photothermal Therapy Nanoplatform by the Assembly of Fe_3O_4-Carbon Dots with Graphitic Black Phosphorus Quantum Dots

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Experimental Section

Synthesis of BPQDs

The BPQDs were prepared using a simple liquid exfoliation technique involving ultrasound probe sonication followed by bath sonication of ground powders of bulk BP. In brief, 50 mg of the BP powder was added in a mortar in batches and then grounded for 2 h per batch in a N_2 glovebox. For fully grinding down into micron-order flour, a small amount of NMP was frequently added into the mortar, and the supernatant mixture was timely transferred to a 200 mL round-bottom flask. Finally, the mixture contained 50 mL of NMP. When the flask was hermetically sealed, it was fetched out from the glovebox. The dispersion was then sonicated in an ultrasonic bath continuously for another 12 h at the power of 200 W. The temperature of sample solution was kept below 277 K by an ice bath. The resulting dispersion was centrifuged for 20 min at 7,000 rpm, and the supernatant containing BPQDs was decanted gently. Then, the BPQDs solution was centrifuged for 20 min at 12,000 rpm, the precipitate was repeated water rinsing and re-suspended in aqueous solutions.

Synthesis of Fe_3O_4-CDs

In a typical synthesis, FAC (0.2 g) and polylysine acid (0.5 g) was dissolved in H_2O. The precursor solution was transferred to a 20 mL Teflon-lined stainless steel

autoclave. After sealing, the autoclave was heated to and maintained at 200 °C for 8 h. The autoclave was then cooled naturally to room temperature. The soliquid was separated by centrifugation (6500 rpm, 2 min) to discard the liquid phase. The obtained solid phase was dispersed in 10 mL acetone by ultrasonic dispersion for 3 minutes, and then separated magnetically. This washing process was repeated three times to remove dissociative CDs and polylysine acid. Finally, the black products were collected by freeze drying for future use. The samples are hereafter denoted as Fe_3O_4-CDs.

Synthesis of PGA-Fe_3O_4-CDs nanoparticles

For synthesis of PGA-Fe_3O_4-CDs, N-(3-dimethylaminopropyl)-N_0-ethylcarbodiimide hydrochloride (EDC, 0.2 g) and N-hydroxysuccinimide (NHS, 0.2 g) were introduced to a methanol suspension (150 mL) of Fe_3O_4-CDs (10 mg) to activate the amino groups of Fe_3O_4-CDs, and the mixture was stirred gently at room temperature for 3 h. Then PGA (0.5 g)was added to the activated Fe_3O_4-CDs solution, followedby another 24 h of stirring. The resulting PGA-Fe_3O_4-CDs was separated by centrifugation, rinsed with methanol repeatedly to remove excess polymer, and then dried under vacuum.

Synthesis of PGA-Fe_3O_4-CDs@BPQDs nanoparticles

Briefly, BPQDs were mixed with the PGA-Fe_3O_4-CDs aqueous solution under continuous stirring. After the pH value was adjusted to about 8.0 with ammonia aqueous solution (0.1×10^{-3} M, 25%) and shaken for 6 h at room temperature, the PGA-Fe_3O_4-CDs@BPQDs composites were obtained with centrifugation. The solid powders were collected through freeze drying for further characterization and use.

Synthesis of GP-PGA-Fe_3O_4-CDs@BPQDs nanoparticles

PGA-Fe_3O_4-CDs@BPQDs (1.8 mg) was dispersed in 1 mL double-distilled water (DDW) to form a homogeneous solution, after which aqueous Genipin (0.5 wt %) was slowly added to the PGA-Fe_3O_4-CDs@BPQDs solution for crosslinking reaction at 60 °C for 5 h to obtain GP-crosslinked PGA-Fe_3O_4-CDs@BPQDs nanoparticles (GP-PGA-Fe_3O_4-CDs@BPQDs). And the solution was filtered through a 0.22 μm membrane to remove large particles. Then, powders were obtained by vacuum

freeze-drying machine. Finally, the powder were rinsed twice with ethyl acetate then freeze-dried and stored in a refrigerator at 4 °C prior to use.

Quantum yield (Φ) measurements

The quantum yield (QY) was a main parameter that used for characterizing the optical conversion efficiency of fluorescent material. Generally, the quinine sulfate as a reference substance was used to measure the quantum yield. Specific experimental procedure is as follows: firstly, a small amount of the quinine sulfate (Φ_R = 54%) was dissolved in 0.1 M solution of H_2SO_4, and the BPQDs and GP-PGA-Fe_3O_4-CDs@BPQDs were dissolved in deionized water. Then, the absorbance values of BPQDs, GP-PGA-Fe_3O_4-CDs@BPQDs and quinine sulfate were measured at 360 nm. Ensuring both of the absorbance values were less than 0.05. In addition, the integral fluorescence intensity of BPQDs, GP-PGA-Fe_3O_4-CDs@BPQDs and quinine sulfate were obtained by measuring both of PL emission spectra at an excitation wavelength of 440 nm. Finally, the quantum yield of BPQDs and GP-PGA-Fe_3O_4-CDs@BPQDs was calculated using following equation:

$$\Phi = \Phi_R \times (K_S / K_R) \times (A_R / A_S) \times (\eta_S / \eta_R)^2$$

Where, Φ and F are the quantum yield and integral fluorescence intensity, A is the absorbance values, η is the refractive index of solvent (both are 1.33). The subscript "R" and "S" denote the reference substance and the samples, respectively.

Photothermal effect of GP-PGA-Fe_3O_4-CDs@BPQDs nanoparticles

1 mL of GP-PGA-Fe_3O_4-CDs@BPQDs (50 μg/mL) aqueous solutions were added into a 3 mL transparent quartz vial. The top of the vial was fixed and a fiber-coupled continuous semiconductor diode laser (660 nm/10 min, 808 nm/5 min) with the power density of 0.5 or 1.0 W/cm^2 was used as the light source to irradiate the bottom of the vial. A thermal infrared imaging camera was used to monitor the temperature change. Photothermal conversion efficiency was evaluated by recording the temperature change of the aqueous dispersion (50 μg/mL) as a function of time under 800 nm laser irradiation at a power density of 2 W/cm^2 until the solution reached a steady-state temperature. Photothermal conversion efficiency, η, was calculated using to the

previous literatures.

$$h = [h{\cdot}A\ (T_{max} - T_{surr}) - Q_0] / [I{\cdot}(1\text{-}1{\times}10^{-A808})]$$

where h is the heat transfer coefficient of the nanocomposites, A is the area cross section of irradiation, Q_0 is the heat dissipated from light absorbed by the quartz sample cell and water (this was measured independently as 25.7 mW using borosilicate glass cells containing water without the addition of the GP-PGA-Fe_3O_4-CDs@BPQDs, I is the incident laser power, A_{808} is the absorbance of the GP-PGA-Fe_3O_4-CDs@BPQDs at 808 nm, T_{max} is the highest temperature that can be reached under irradiation, and T_{surr} is the surrounding temperature. The value of $h{\cdot}A$ can be derived from the following eq:

$$\tau_s = [\sum m_i\ C_{p,i}\ ] / [h{\cdot}A]$$

where mi and $C_{p,i}$ are the mass of and heat capacity of the irradiated system, respectively, including water, the quartz cell, and the GP-PGA-Fe_3O_4-CDs@BPQDs. The values for mi are 0.5 and 5.7 g, while the values for $C_{p,i}$ are 4.2 and 0.89 J/(g·K) for water and the quartz cell, respectively. The time constant, τ_s, is defined as the slope of cooling time against $-\ln(\theta)$, where θ is the temperature driving force, which is defined by eq:

$$\theta = [T_{surr} - T] / [T_{surr} - T_{max}]$$

where T is the temperature of the system.

ROS assay

HeLa cells were seeded into six-well plate with the cell number of 2×10^5 cells per well. After 24 h, different concentrations of GP-PGA-Fe_3O_4-CDs@BPQDs were added into the cells and incubated for additional 3 h under serum-free conditions. The cells were washed with PBS and replaced with serum containing medium along with DCFH-DA solution (5×10^{-6} M in cell culturemedium) were added and incubated for 30 min at 37 °C. As a positive control, 5×10^{-6} M H_2O_2 was also added to the cells and incubated for 30 min. Cells were then trypsinized and aspirated, followed by flow cytometry analysis (BD FACS Canto) equipped with 488 nm laser. Green fluorescence was monitored using FITC channel.

Evaluation of the $^1O_2$ quantum yield by detecting its emission

Measurement of $^1O_2$ Quantum Yield of GP-PGA-Fe_3O_4-CDs@BPQDs by a Physical Method: According to a previous report, the $^1O_2$ emission was detected in a FluoroSENS fluorescence spectrophotometer with a NIR detector at 660 nm excitation using MB as a reference standard. In view of the short PL lifetime of $^1O_2$ in water, the GP-PGA-Fe_3O_4-CDs@BPQDs solution was lyophilized as solid powder first, and then redissolved in CD_3OD-D_2O mixture solution (v/v = 10/1). The absorptions of MB and the GP-PGA-Fe_3O_4-CDs@BPQDs at 660 nm were regulated to ≈0.1 OD. The $^1O_2$ quantum yield of GP-PGA-Fe_3O_4-CDs@BPQDs could be determined using equation:

$$\Phi_0 = \Phi_{MB} \times (I_0 / I_{MB})$$

Where I_0 and I_{MB} indicate the PL peak areas of $^1O_2$ generated from the GP-PGA-Fe_3O_4-CDs@BPQDs and MB, respectively. Φ_{MB} is the $^1O_2$ quantum yield in CD_3OD, which is given as 0.58.

Hemolysis assay

Human blood was centrifuged at 3000 rpm and washed five times with PBS solution to pure erythrocytes. Then, 0.15 mL of 4% erythrocytes (v/v) was mixed with 0.15 mL of water, saline or GP-PGA-Fe_3O_4-CDs@BPQDs solution at various concentrations. The mixtures were incubated at 37 °C for 8 h. After spinning down the erythrocytes, the supernatants were collected, and the absorbance at 540 nm was recorded using a UV-vis spectrophotometer. The percentage of hemolysis was calculated using equation:

$$\text{Hemolysis} = (I / I_0) \times 100\%$$

where I is the absorbance of erythrocytes with different concentrations of GP-PGA-Fe_3O_4-CDs@BPQDs, and I_0 is the absorbance of the complete hemolysis in distilled water.

Morphological Changes of RBCs

For observing morphological changes of treated RBCs at the early stages of hemolysis, the GP-PGA-Fe_3O_4-CDs@BPQDs (0-100 μg/mL) were diluted to the required concentrations in RBC suspensions. The cell pellets obtained after 1.5 h by centrifugation, were diluted in PBS and mounted on clean glass slides covered with

coverslips and observed under an Olympus BX41 microscope with a camera (Olympus E-620, Olympus Ltd., Japan).

Cell imaging

For the cell imaging experiment, (human liver carcinoma cells) HepG2 cells were seeded in each well of 6-well sterile chamber slides in a 500 μL culture medium at a cell density of 1×10^4 cells/well. Following overnight incubation, the media was replaced with a culture media containing 10 μg/mL GP-PGA-Fe_3O_4-CDs@BPQDs and incubated for 2 h. The cells monolayer was washed three times with phosphate-buffered saline (PBS, PH = 7.4) and fixed on a solid with 3% paraformaldehyde for 10 min. The cells were then imaged using a laser scanning confocal microscope.

Assay of cell viability

The C6, HeLa, A549 and MCF7 cells were obtained from China type culture collection (CTCC). These cells were cultured on a 96-well plate (1×10^4 cells/well) in Dulbecco's Modified Eagle medium (Gibco BRL) supplemented with 10 % (v/v) fetal bovine serum, 100 UI/mL penicillin and 100 UI/mL streptomycin. The cells were incubated in a humid chamber of 5 % CO_2 at 37 °C. After 12 h, the DMEM was replaced with 200 μL of the DMEM medium that contained 20 μL/well of the desired amount of samples (GP-PGA-Fe_3O_4-CDs@BPQDs with different concentrations of 6, 12, 24, 48, 96 μg/mL). Five multiple holes were set for every sample. The cells were treated with samples for 48 h. MTT was dissolved in PBS solution at concentration of 5 mg/mL and filtered through a 0.22 μM filter to sterilize and remove insoluble residues, then stored in the amber vials at 4 °C. The cell viability was assayed by adding 20 μL of MTT PBS solution (5 mg/mL) to each well. After the cells were incubated with MTT at 37 °C 4 h, the MTT solution was removed from all samples and 200 μL of DMSO was added to dissolve the eventually formed formazan crystals. The absorbance that correlated with the number of viable cells in each well was measured by a Thermo Reader with the wavelength of 490 nm. The following formula was used to calculate the inhibition of cell growth: Cell viability (%) = (mean of Abs. value of treatment group/mean Abs. value of control)×100%.

Assay of cell apoptosis and necrosis

To detect cell apoptosis and necrosis, Annexin V-FITC/PI apoptosis assay (TransGen Biotech) was involved. In brief, C6, HeLa, A549 and MCF7 cells at a density of 1.0×10^5 cells per mL were seeded onto the 24 well-plate and cultured overnight. Subsequently, the cells were treated with 50 μg/mL GP-PGA-Fe_3O_4-CDs@BPQDs for 24 h. Finally, the cells were harvested, rinsed thrice with PBS, stained with Annexin V-FITC and PI (Annexin V-FITC labeled apoptotic cells while PI labeled necrotic cells), and then analyzed by a cell Lab Quanta SC flow cytometry (Beckman coulter, USA).

***In vivo* toxicity studies of GP-PGA-Fe_3O_4-CDs@BPQDs nanoparticles**

The healthy male C57BL/6 mice (body mass 20 g) were provided by Nanjing University Animal Center, Nanjing, China. All animal operations were conducted in accordance with the guidelines of the Institutional Animal Care and Use Committee. The material dispersed in PBS (pH 7.4, 0.1 mL) at a total dose of 10 mg/kg, was intravenously injected into the mice (3 KM mice per group) via the tail vein. C57BL/6 mice (n = 3) receiving the injection of only physiological saline were chosen as the control group. Tissues were harvested from mice 5 and 30 days post-injection. The organs (heart, liver, spleen, lung and kidneys) were removed and fixed in 4% formaldehyde, then dehydrated and embedded in paraffin. 5 mm transverse sections were cut (Leica RM2235 microtome) and stained with HE for histological analysis. The organs were excised and weighted. Organ index (in g/g) was calculated from the ratio of the wet weight of the individual organ to the whole body weight. As collected above, the organs of GP-PGA-Fe_3O_4-CDs@BPQDs-treated mice were digested using a microwave system. The phosphorus content was measured by an inductively coupled plasma mass spectrometer.

Blood circulation

100 μL of GP-PGA-Fe_3O_4-CDs@BPQDs (50 μg/mL) was injected via the tail vein into each mouse. At various time points about 20 μL of blood was sampled from each mouse and dissolved in 80 μL of lysis buffer (1% SDS, 1% Triton X-100, 40 mM Tris acetate, 10 mM EDTA, 10 mM DTT). Raman spectra of the lyzed blood

samples were recorded to determine BPQDs levels in the blood based on the characteristic Raman signals from BPQDs (435 cm^{-1}). The percent injected dose per gram (%ID/g) of blood was calculated by the following equation:

$$\%ID/g = [BPQDs]_{bloodlysate} \times V_{bloodlysate} /([BPQDs]_{injected} \times V_{injectedBPQDs} \times bloodweight) \times 100\%$$

***In vivo* hematology studies of GP-PGA-Fe_3O_4-CDs@BPQDs**

The healthy female C57BL/6 mice (body mass≈20 g) were provided by Nanjing University Laboratory Animal Center. All animal operations were conducted in accordance with the guidelines of the Institutional Animal Care and Use Committee. The material dispersed in normal saline (100 μL) at a total dose of 10 mg/kg was intravenously injected into the mice (3 mice per group) via the tail vein. C57BL/6 mice (n =3) receiving the injection of only physiological saline were chosen as the control group. Blood samples and tissues were harvested from mice 5 days and 30 days post-injection. Blood was collected from the orbital sinus by quickly removing the eyeball from the socket with a pair of tissue forceps. For serum biochemistry assays, four important indicators (alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin (TB) and creatinine (Cr)) were determined.

***In vitro* ROS experiments**

HeLa cells (2×10^4 cells/mL) were cultured in a 24-well plate and incubated for 1 d to allow cells to stick onto the surface of the plate. Different concentrations of GP-PGA-Fe_3O_4-CDs@BPQDs were added to the 24-well plate and the cell solutions were incubated in dark at 37 °C for 3 h. To determine the phototoxicities of GP-PGA-Fe_3O_4-CDs@BPQDs, HeLa cells were irradiated with 660 nm laser (0.5 W/cm^2, 30 min). After photoirradiation, the cells were trypsinized and aspirated, followed by flow cytometry analysis by monitoring the green fluorescence using the FITC channel. For NaN_3 quenching experiments, HeLa cells were pretreated with 50×10^{-3} M NaN_3 solution mixed in PBS and incubated for 1 h, followed by photoirradiation and subjected to ROS analysis.

***In vitro* DNA damage quantification**

The evaluation of DNA damage was performed by a revised protocol of comet assay.

Agarose (0.8%, 500 μL) was coated homogeneously on glass microscopic slides. After solidification, 4.8 × 10^4 cells in 30 μL of PBS incubated with GP-PGA-Fe_3O_4-CDs@BPQDs (50 μg/mL) were mixed with the agarose (0.6%, 70 μL), and 20 μL of this mixture was spread over the slide completely. The solidified slides were placed into the newly prepared ice lysis buffer solution (1% Triton X-100, 10% DMSO, 100 mmol/L of Na_2EDTA, 10 mmol/L of Tris-HCl, and 2.5 mol/L of NaCl) for 2 h and then immersed into a horizontal gel electrophoresis unit that was filled with chilled electrophoresis buffer (pH 7.4, 300 mmol/L of NaOH, 1 mmol/L of Na_2EDTA) for 30 min. After electrophoresis for 20 min at 30 V, the slides were neutralized with ethyl alcohol and stained with ethidium bromide (2 μg/mL). Finally, the Comet Assay Software Project was adopted to analyze the tail DNA and tail moments by counting 100 cells.

***In vitro* antitumor efficiency of GP-PGA-Fe_3O_4-CDs@BPQDs nanoparticles**

To determine the PTT or PDT efficacy of GP-PGA-Fe_3O_4-CDs@BPQDs, the standard MTT assay was carried out to determine the relative viabilities of cancer cells with various treatments. The cell viability was normalized by control group without any treatment. HeLa and C6 cells (1×10^4 cells/well) were seeded in 96-well plates and incubated overnight at 37 °C in a humidified 5% CO_2 atmosphere. After rinsing with PBS (pH 7.4), the HeLa and C6 cells were incubated with and without GP-PGA-Fe_3O_4-CDs@BPQDs (25 μg/mL) at 37 ºC under the same conditions. After being cultured for 4 h, the cells in PTT therapy group were irradiated with 808 nm laser (2 W/cm^2) for 5 min. For PDT therapy, the cells were irradiation with 660 nm laser (0.5 W/cm^2) for 10 min. To determine the cytotoxicity of NIR light, the cells were irradiated under 808 and 660 nm lasers for 5 and 10 min, respectively. The laser spot was adjusted to fully cover the area of each well. After illumination, the cells were incubated for 12 h in a 5% CO_2, 95% air humidified incubator at 37 °C. Afterwards, the treated cells were co-stained with Calcein AM and PI for 30 min. Afterward, the cells of experimental group were rinsed again with PBS and then imaged by an Olympus IX71 motorized inverted microscope.

***In vivo* T_2-weight MRI**

Female C57BL/6 mice (5 weeks old) were purchased from the Nanjing University Laboratory Animal Center. Firstly, the mice were anesthetized using 5% chloral hydrate (10 mL). Subsequently, 0.5 mg of GP-PGA-Fe_3O_4-CDs@BPQDs, dispersion solution was injected through the tail vein into the mouse. *In vivo* multi-imaging was performed at appropriate time points after tail vein injection. All MRI studies were performed on a 9.4 T/400 mm wide bore scanner (Agilent Technologies, Inc., Santa Clara, CA, USA), using a volume RF coil. T2-weighted FSE-XL/90 sequence with the following parameters: repetition time (TR)=5000 ms, TE=48 ms: field of view [FOV]: 30 mm×30 mm; matrix: 256×256; number of excitations (NEX): 5; slice thickness=1 mm (12 slices, gap=0), and bandwidth (BW)=25 kHz.; echo spacing=10 ms; echo train length (ETL)=8; effective echo time (TE)=30 ms; 2 averages. Across scanning sessions, precise measurements and markers were used to ensure consistent placement of the mice's tumor in the animal holder, and the mice's tumor within the magnet. Pulse oximeter triggering was used for the MRI acquisition to reduce artifacts arising from respiratory movement.

***In vivo* imaging and biodistribution**

In the biodistribution and imaging, fluorescent labelled BPQDs/PLGA NSs were prepared by adding 0.1 mg/mL of Cy5.5 to the GP-PGA-Fe_3O_4-CDs@BPQDs solution in DCM followed by oil-in-water emulsion solvent evaporation. The excess dye molecules were removed by centrifugation and washed away with water more than 5 times until no noticeable colour change was observed from the supernatant fluids followed by resuspension in PBS.

Female Nude mice (5 weeks old, 15-20 g) were purchased from model animal research central of Nanjing university and used under regulation approved by the Laboratory Animal Center of the Nanjing normal univeristy. In the subcutaneous injection experiments, a subcutaneous HeLa tumor was established by injecting a suspension of 2×10^6 HeLa cells in PBS (200 μL) into the shoulder of each female nude mouse and was allowed to grow for 8–10 d when the tumor size reached from 50 mm^3. Cy5.5-labelled GP-PGA-Fe_3O_4-CDs@BPQDs (1 mg/mL, 100 μL) were subcutaneously injected into the tumor of mice. Fluorescent scans were performed at

various time points (0-24 h) postinjection using a Maestro 2 Multispectral Small-animal Imaging System. The tumor-bearing mice were sacrificed by exsanguinations at 24 h post-injection, and the tumor and major organs were harvested. As collected above, the organs of Cy5.5-labelled GP-PGA-Fe_3O_4-CDs@BPQDs-treated mice were digested using a microwave system (CEM Mars 5, Kamp Lintfort, Germany). The phosphorus content was measured by an inductively coupled plasma mass spectrometer (ICP-MS, Agilent 7500 CE, Agilent Technologies, Waldbronn, Germany).

***In vivo* antitumor efficiency GP-PGA-Fe_3O_4-CDs@BPQDs nanoparticles**

Animal experiments were approved by the China Committee for Research and Animal Ethics in compliance with the law on experimental animals. The tumor model was generated by subcutaneously injecting 200 μL of normal saline containing 1×10^6 HeLa cells into the back of each mouse. When the tumor volume reached about 100 mm^3, the mice were divided into five groups (n=5 in each group): (i) saline, (ii) GP-PGA-Fe_3O_4-CDs@BPQDs, (iii) GP-PGA-Fe_3O_4-CDs@BPQDs with 660 nm irradiation, (iv) GP-PGA-Fe_3O_4-CDs@BPQDs with 808 nm irradiation, (v) GP-PGA-Fe_3O_4-CDs@BPQDs with 660 and 808 nm irradiation. For group (ii-v), the mice were received an intratumoral injection of 100 μL of GP-PGA-Fe_3O_4-CDs@BPQDs (50 μg/mL), respectively. For the PTT treatment, after 2 h injection, the mice were irradiated by the 808 nm laser at the power density of 1 W/cm^2 for 5 min. While for PDT treatment, after 2.5 h injection, the mice were irradiated by the 660 nm laser at the power density of 0.5 W/cm^2 for 15 min. During the irradiation, the local temperature of tumor were monitored using thermal infrared imaging camera. The tumor volume and body weight were recorded every other day. Fourteen days later, the mice were sacrificed and the tumor, heart, liver, spleen, lung and kidney were excised for histological section and imaging. When the experiments were finished, the tumors were removed, and their volume was estimated (V, mm^3) using the following formula: $V = a \times b^2/2$, where a is the length and b is the width in millimeters.

Statistical analysis

Data in this study were presented as mean ± SEM. Each experiment was performed in triplicate. Statistical significance was determined by one-way analysis of variance/Student's t-test. P-values of < 0.05 were considered significantly different.

Figure Section

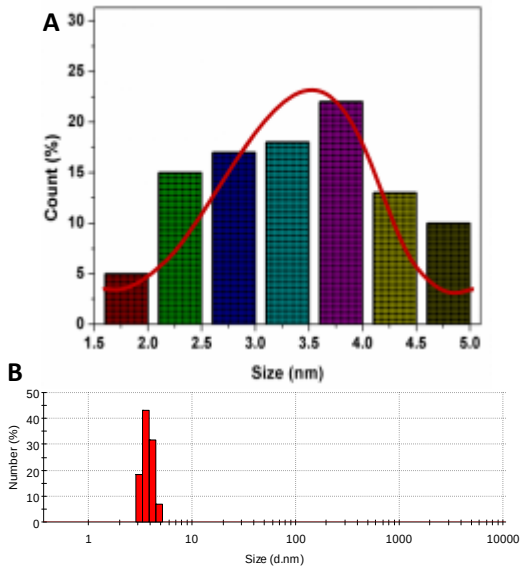

Fig. S1. Statistical analysis of the lateral size of the 100 BPQDs based on TEM.

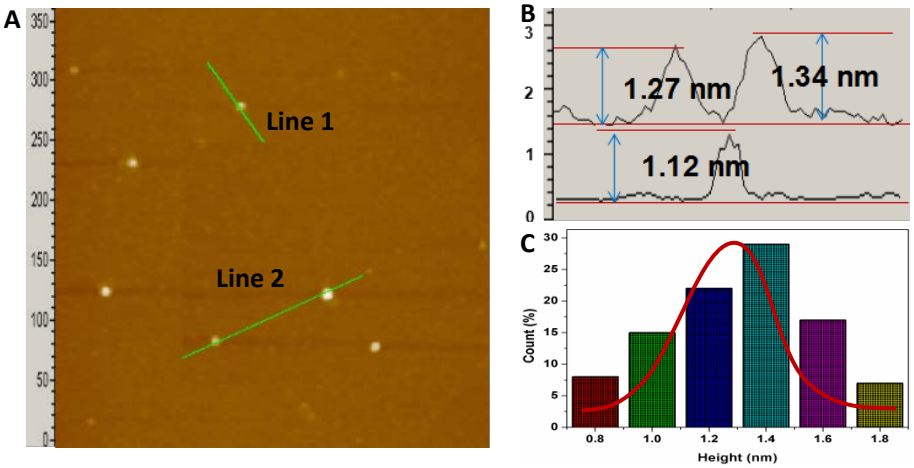

Fig. S2. (A) AFM image. (B) Height profiles along the black lines in (A). (C) Statistical analysis of the heights of 50 BPQDs determined by AFM.

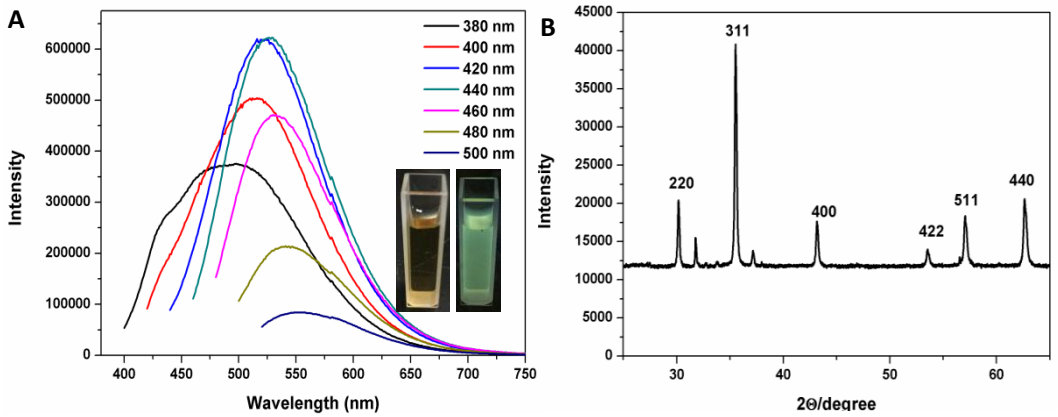

Fig. S3. (A) Emission spectra of the BPQDs with excitation of different wavelength (from 380 to 500 nm). Inset: photographs of the solution of BPQDs under visible light (left) and 365 nm UV light (right). (B) XRD patterns of the synthesized Fe_3O_4-CDs.

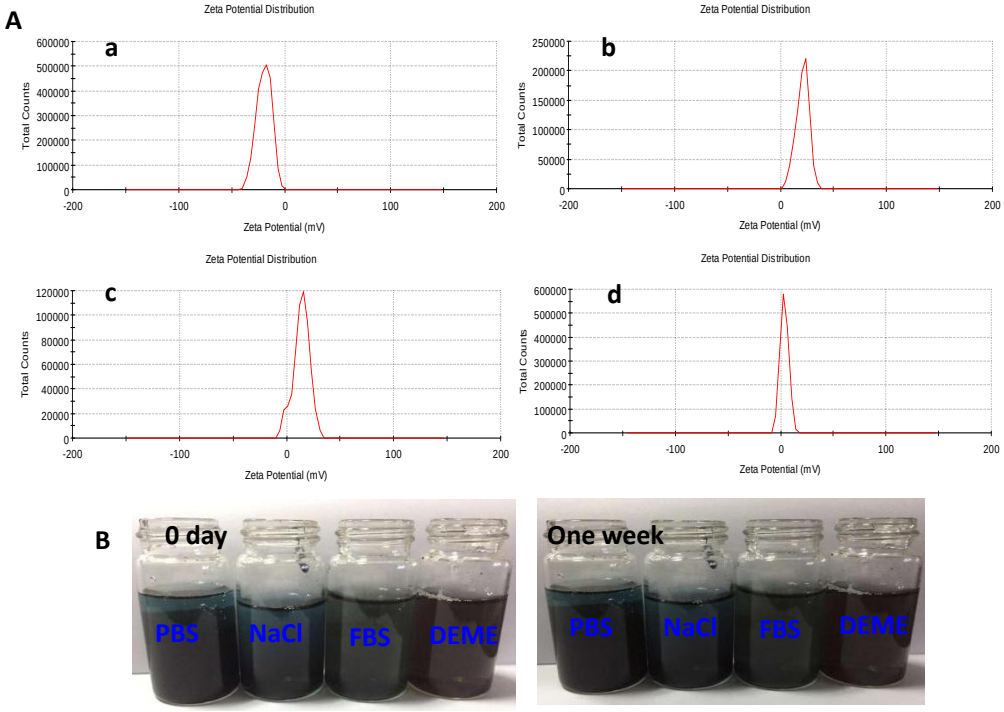

Fig. S4. (A) zeta potentials of BPQDs (a), Fe3O4-CDs (b), PGA-Fe3O4-CDs (c) and PGA-Fe3O4-CDs@BPQDs (d). (B) Images of GP-PGA-Fe3O4-CDs@BPQDs nanoparticles in different media for one week.

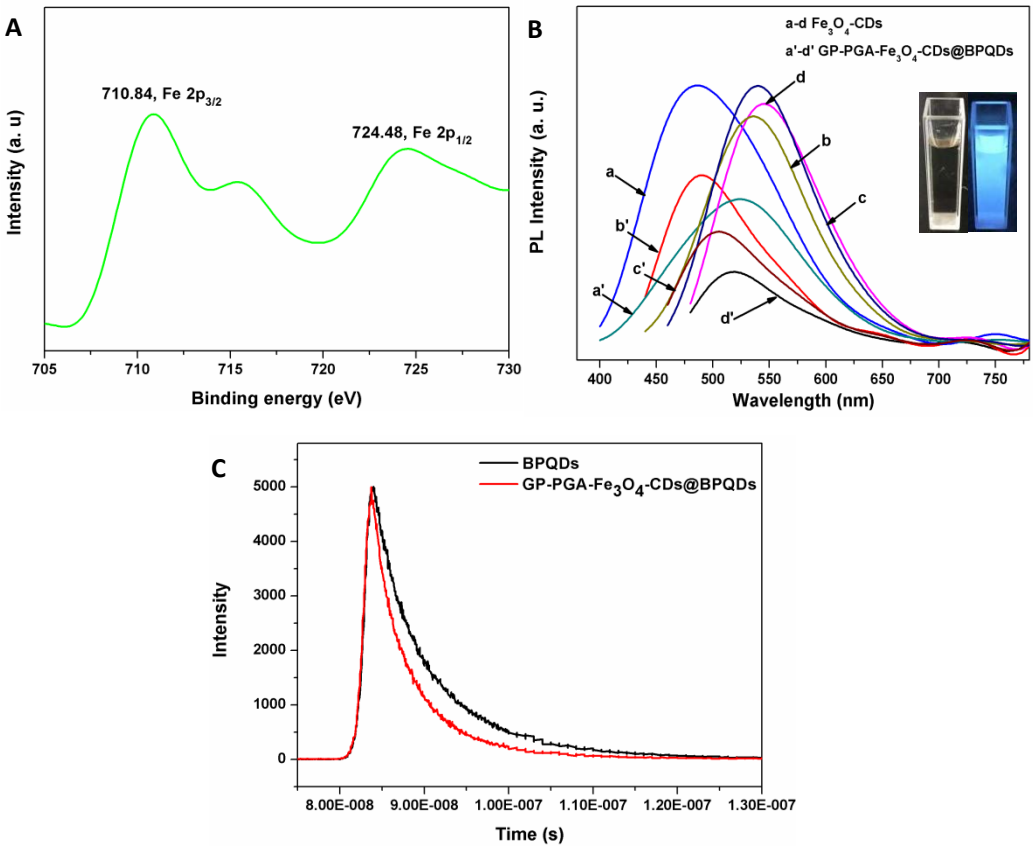

Fig. S5. (A) Fe 2p spectra of GP-PGA-Fe_3O_4-CDs@BPQDs nanoparticles. (B) Emission spectra of the Fe_3O_4-CDs and GP-PGA-Fe_3O_4-CDs@BPQDs with excitation of different wavelength (380, 420, 440 and 460 nm). Inset: photographs of the solution of GP-PGA-Fe_3O_4-CDs@BPQDs under visible light (left) and 365 nm UV light (right). (C) A typical time-resolved fluorescence decay curve of the BPQDs and GP-PGA-Fe_3O_4-CDs@BPQDs was monitored under the emission at 520 nm when excited at 440 nm.

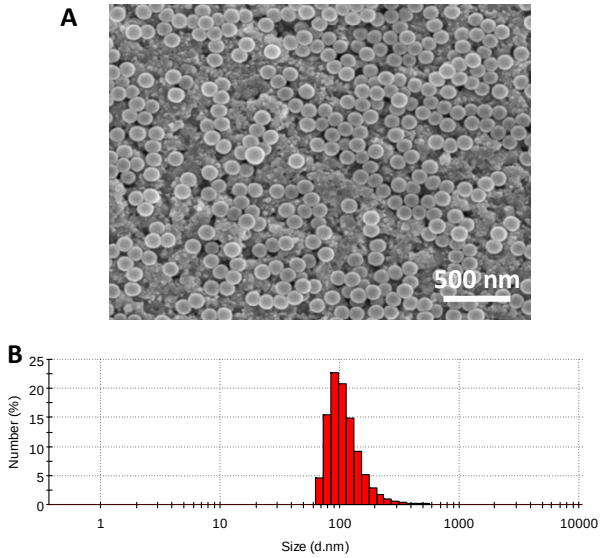

Fig. S6. (A) SEM image of GP-PGA-Fe_3O_4-CDs@BPQDs nanoparticles. (B) DLS measurements of GP-PGA-Fe_3O_4-CDs@BPQDs nanoparticles.

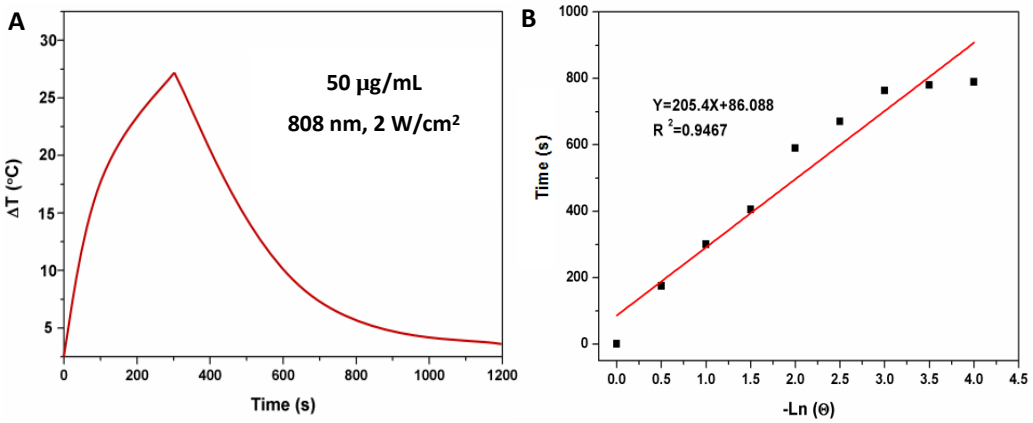

Fig. S7. (A) The photothermal response of GP-PGA-Fe_3O_4-CDs@BPQDs solution irradiated under an 808 nm laser at 2 W/cm^2 for 300 s, then the laser was turned off. (B) Linear relationship between time and lnθ obtained from the cooling time of (A).

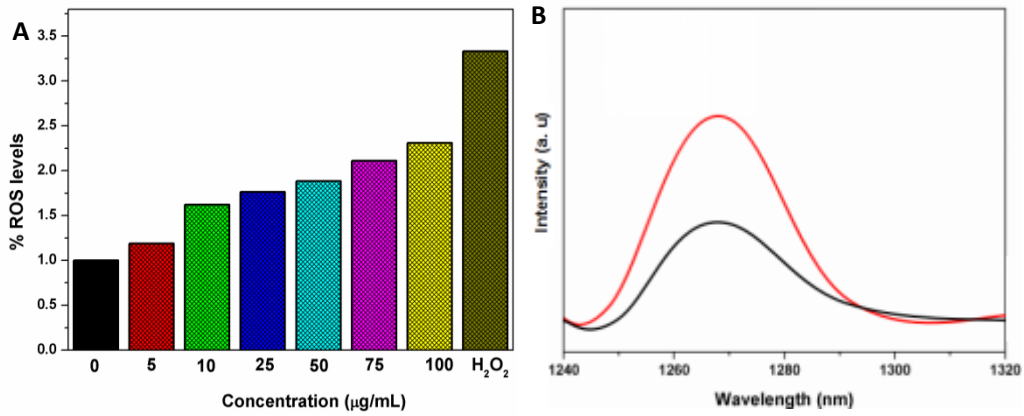

Fig. S8. (A) ROS generation monitored for various concentrations of GP-PGA-Fe_3O_4-CDs@BPQDs nanoparticles in HeLa cells for 3 h and the mean fluorescence intensities of DCF were quantified. Pretreatment of cells with 10×10^{-6} M H_2O_2 was served as a positive control, respectively. (B) $^1O_2$ emissions at ≈1270 nm induced by GP-PGA-Fe_3O_4-CDs@BPQDs nanoparticles and MB in a CD_3OD-D_2O mixture solution (v/v = 10/1) under excitation at 660 nm laser (0.5 W/cm^2).

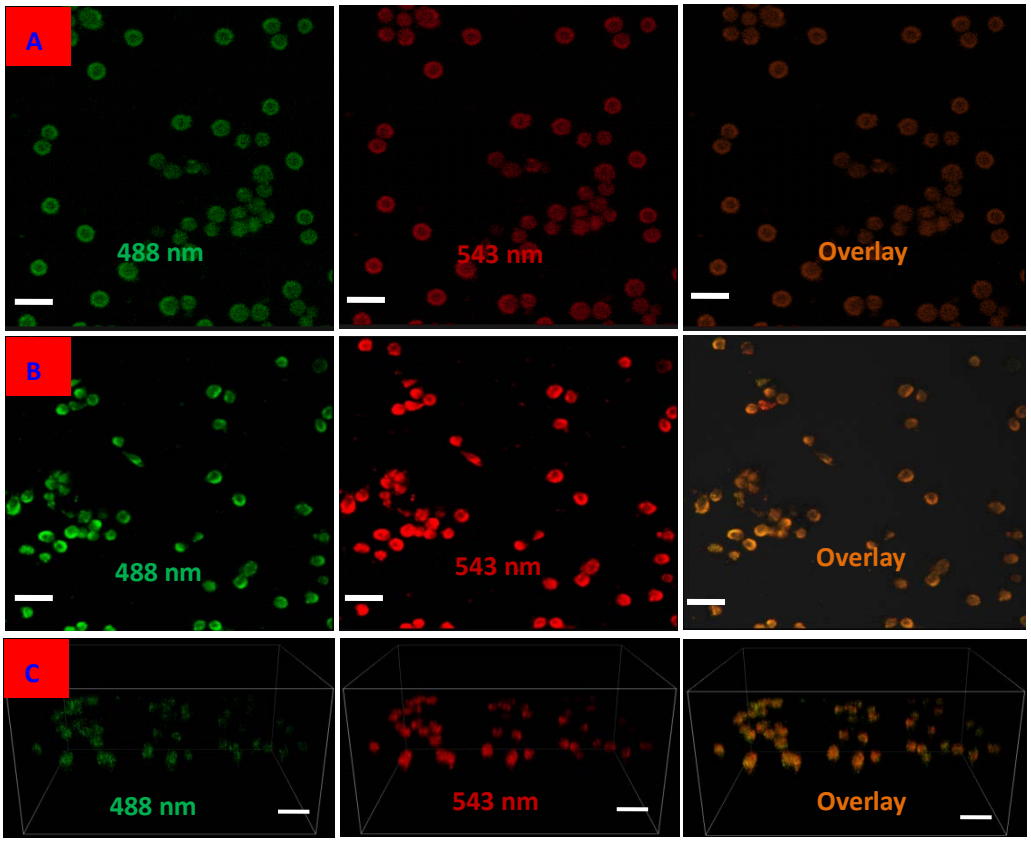

Fig. S9. *In vitro* confocal microscopic imaging of HepG2 cells 6 h (A) and 12 h (B) cultured with GP-PGA-Fe_3O_4-CDs@BPQDs nanoparticles. (C) 3D z-stack images of 3D cultured HepG2 cells 6 h cultivated with GP-PGA-Fe_3O_4-CDs@BPQDs. Scale bar = 50 µm.

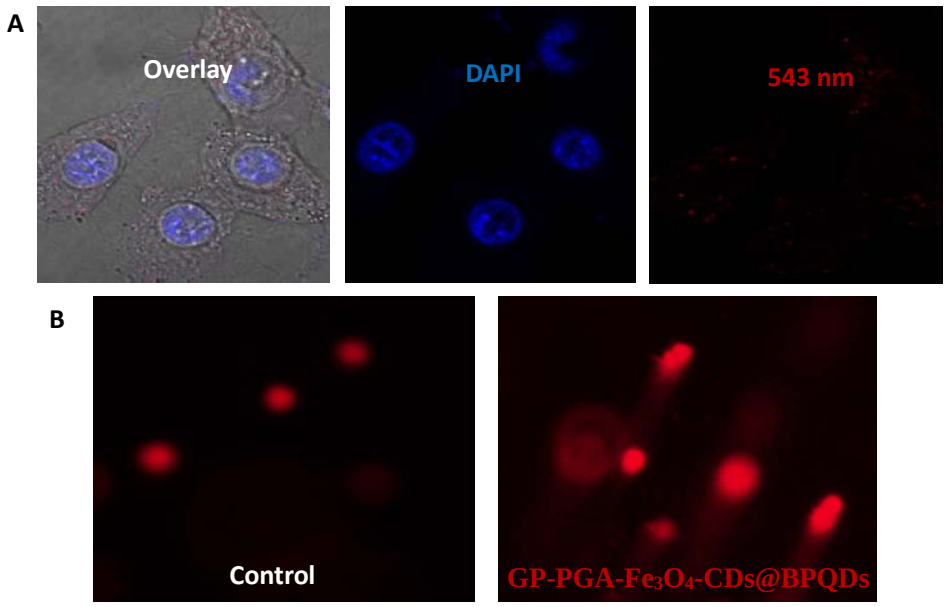

Fig. S10. (A) Confocal fluorescence images of HepG2 cells without GP-PGA-Fe_3O_4-CDs@BPQDs. (B) *In vitro* images of comet assays treated with or without GP-PGA-Fe_3O_4-CDs@BPQDs.

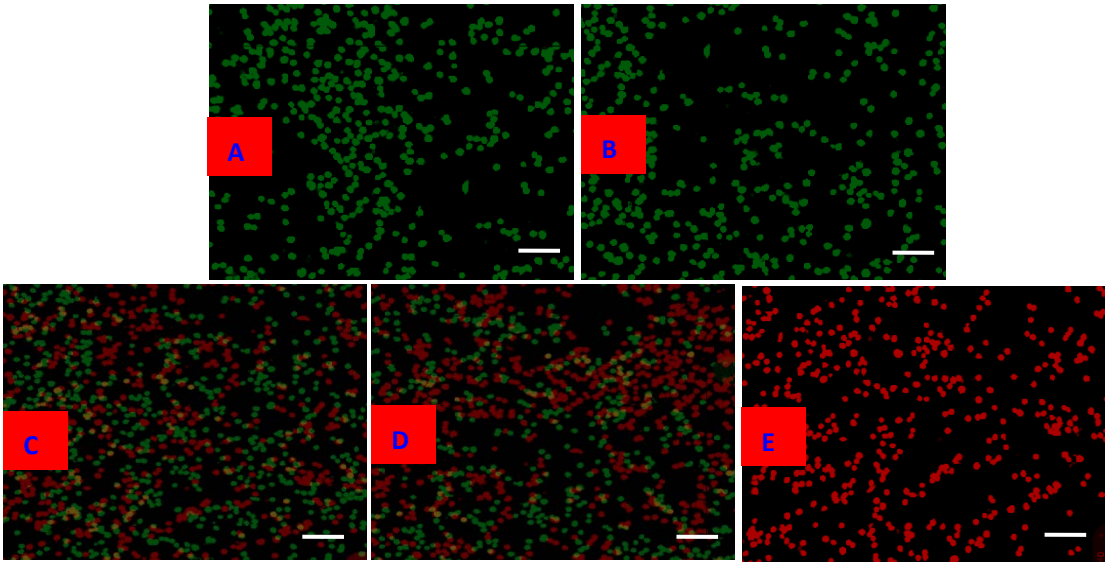

Fig. S11. Fluorescence images of calcein AM/PI-stained HeLa cells incubated with various media: (A) laser only (0.5 W/cm^2), (B) laser only (2 W/cm^2), (C) 50 μg/mL of GP-PGA-Fe_3O_4-CDs@BPQDs nanoparticles + laser (660 nm, 0.5 W/cm^2, PDT), (D) 50 μg/mL of GP-PGA-Fe_3O_4-CDs@BPQDs nanoparticles + laser (808 nm, 2 W/cm^2, PTT), and (E) 50 μg/mL of GP-PGA-Fe_3O_4-CDs@BPQDs nanoparticles + laser (660+808 nm, PDT/PTT). Scale bar = 100 μm.

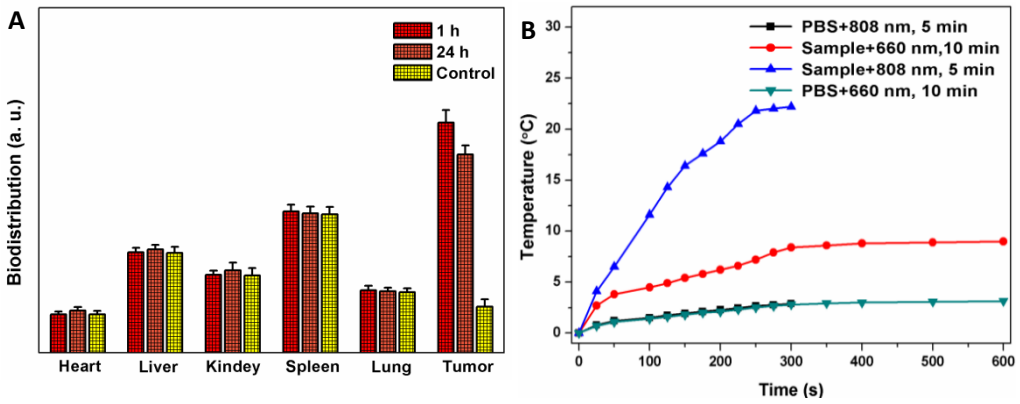

Fig. S12. (A) Biodistribution of P in major organs of mice without or with GP-PGA-Fe_3O_4-CDs@BPQDs injection at different time points postinjection. (B) The temperature variation on tumors of mice with and without GP-PGA-Fe_3O_4-CDs@BPQDs injection after the 808 nm laser irradiation at 2 W/cm^2 or 660 nm laser irradiation at 0.5 W/cm^2.

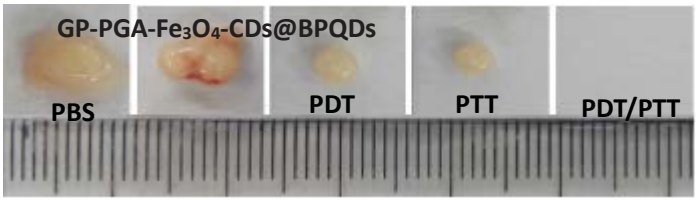

Fig. S13. Digital photo of representative tumor in mice with different treatments.

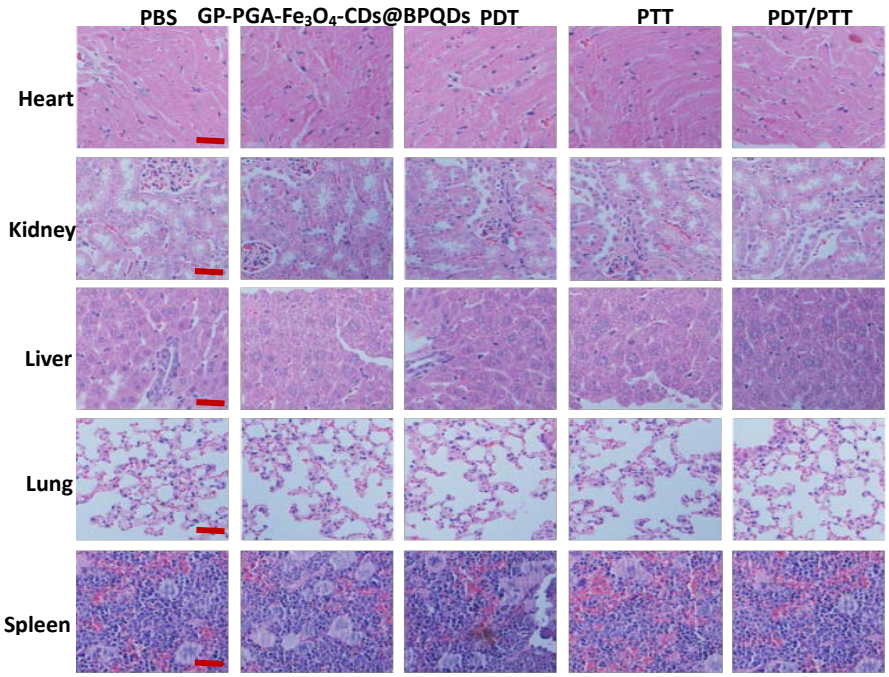

Fig. S14. Hematoxylin and eosin (H&E)-stained slices of the heart, liver, spleen, lung, and kidney in mice after therapy. Scale bar = 50 μm.