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Supporting Information

Boosting Cancer Therapy Efficiency via Photoinduced Radical Production

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MATERIALS AND METHODS

Materials

Pentafluoro benzaldehyde and pyrrole were purchased from Macklin Reagents (Shanghai). *p*-Toluenesulfonyl hydrazide was purchased from J&K Scientific Ltd (Shanghai, China). 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) and 4-dimethylaminopyridine (DMAP) were obtained from Aladdin Reagents of China. Dichloromethane (DCM) and *N*, *N*-dimethylformamide (DMF) were dried over with calcium hydride (CaH_2) and distilled before use. Poly(ethylene glycol)-*block*-poly(D,L-lactide) (PEG-*b*-PLA) were obtained according to the previous literature.[1] All other chemicals were utilized as received without further purification.

Measurements

^1H NMR spectra in $CDCl_3$ were determined by a Bruker AVANCE III HD 400 Spectrometer with tetramethylsilane as the internal standard. The UV-Vis spectra of the samples were measured over different irradiation time intervals using a Thermo Scientific Evolution 220 spectrophotometer. Dynamic light scattering was performed on an Anton-Paar Litesizer™ 500 particle analyzer at room temperature. TEM samples were prepared by dropping the nanoparticles solution (1 mg/mL) on to a carbon coated copper grid, and the images were observed on a JEOL JEM1400 electron microscope operated at 100 kV.

Methods

Synthesis of TFPP

Pyrrole (268 mg, 4 mmol) and pentafluoro benzaldehyde (784.3 mg, 4 mmol) were dissolved in 300 mL dry dichloromethane in a 500 mL flask and then degassed with nitrogen for 30 min. Activated molecular sieve (10 mL) and boron trifluoride etherate (47% solution in diethyl ether, 0.3 mL, 1.12 mmol) were added subsequently. The mixture was shielded from ambient light. After stirring at room temperature for 4 h, 1 g (4.06 mmol) of *p*-tetrachlorobenzoquinone was added to the solution and the mixture was heated to reflux at 50 ℃ for 1 h. Then the solution containing the product was obtained by filtration under reduced pressure and concentrated by rotary evaporation. The crude product was further purified by column chromatography (dichloromethane: petroleum ether = 1:4) to obtain TFPP (400 mg, yield: 41%).

Synthesis of FBC

TFPP (250 mg) and 1.4 g of *p*-toluenesulfonyl hydrazide were carefully mixed and then kept under vacuum for 1 h. Subsequently, the mixture was heated to 158 ℃ for 15 min. After cooling to room temperature, the mixture was purified by silica gel chromatograph (dichloromethane: petroleum ether = 90 :10) to obtain **FBC**.

Synthesis of 2-Iodoethyl Methacrylate (IEMA)

Methacryloyl chloride (3 mL, 31 mmol) and 2-iodoethanol (1 mL, 12.8 mmol) were dissolved in dry dichloromethane (10 mL) under a nitrogen atmosphere. Then triethylamine (4.5 mL, 32 mmol) was added into the mixture and the mixture was stirred at room temperature for 4 h. The mixture was diluted by dichloromethane and washed with saturated sodium bicarbonate solution. Finally, the product-containing dichloromethane solution was dried over anhydrous sodium sulfate. 2-Iodoethyl methacrylate was obtained after removing the solution under vacuum.

Synthesis of POEGMA-*b*-PIEMA Block Copolymer (PI)

POEGMA homopolymer was first synthesized according to a previously reported procedure.[2] Thereafter, 100 mg POEGMA homopolymer (10 μmol), 2-iodoethyl methacrylate (0.35 mL, 2.15 mmol), AIBN (0.1 mL, 3 μmol) and 1 mL anhydrous THF were charged in a reaction vial equipped with a magnetic stir bar. The mixture was degassed by several freeze-thaw cycles and sealed in vacuum. Then the reaction was carried out in a preheated oil bath at 70 ℃. After 5 h, the flask was plunged into liquid nitrogen. The solution was transferred to a dialysis bag (MWCO, 8000-14000) and dialyzed for 72 h against a mixture of methanol/water (1:4, v/v). Finally, the product-containing solution was frozen and lyophilized under vacuum to afford POEGMA-*b*-PIEMA block copolymer. $M_{n,\,NMR}$ = 10,700 g/mol, $M_{n,\,GPC}$ = 9, 700 g/mol, M_w/M_n = 1.15.

Self-Assembly of POEGMA-*b*-PIEMA Block Copolymer

POEGMA-*b*-PIEMA (20 mg) and **FBC** (0.5 mg) were dissolved in 5 mL of dimethyl formamide. Under vigorous stirring, 1 mL of the mixed solution was added dropwise to deionized water (9 mL) at room temperature. After stirring for 1 h, the organic solvent was removed by dialysis against deionized water for 24 h using a dialysis membrane (MWCO = 3500). The **FBC**-encapsulated POEGMA-*b*-PIEMA block copolymer nanoparticles (**PI/FBC**) were obtained. **PC/FBC** and **PI** nanoparticles were also prepared in a similar way as control samples.

ROS Production

As a ROS scavenger, 1, 3-diphenylisobenzofuran (DPBF) was used to determine the ROS production of nanoparticles. A solution containing a fixed concentration of **PI/FBC** nanoparticles and DPBF was added into a quartz cuvette and irradiated at 750 nm for 50 s. The ROS generation of nanoparticles can be directly correlated with the decrease of the DPBF absorbance in the UV-vis spectrum, thus the absorbance of DPBF at 425 nm was measured every 5 s. **PC/FBC** nanoparticles at the same photosensitizer concentration were tested as the control.

Free Radical Production

2,2'-Amino-di(2-ethyl-benzothiazoline sulphonic acid-6) ammonium salt (ABTS) as a radical probe was adopted to evaluate the production of radicals. Briefly, **PI/FBC** and **PC/FBC** nanoparticles were mixed with ABTS at the same **FBC** concentration, respectively. Then, 750 nm laser was used to irradiate the solution for 10 min. The absorbance of ABTS was detected before and after irradiation for observing the production of radicals.

A radical trap agent TEMPO was also utilized to capture free radicals for confirming the radical formation by electron transfer from **FBC** to iodine compound upon illumination. FBC, iodoethane and TEMPO were dissolved in DMSO, then after degassing by N_2, 750 nm laser were employed to illuminate the mixture for 1 h. Samples were taken every 0.5 h and dissolved in $CDCl_3$ for ^1H NMR detection.

Cell Culture

4T1 murine breast cancer cells were cultivated in Dulbecco's modified Eagle's medium (DMEM) supplemented with 1% antibiotics (penicillin and streptomycin) and 10% fetal bovine serum (FBS) in a humidified standard atmosphere of 5% CO_2 at 37 ℃.

Intracellular Reactive Species Generation Assay

The intracellular reactive species generation was measured by using dichlorofluorescein diacetate (DCFH-DA) as a probe. The DCFH-DA could be oxidized into highly fluorescent DCF by reactive species. Typically, 4T1 cells with a density of 1×10^5 containing complete DMEM media were incubated in 6-well plates for 24 h. Then, the 4T1 cells were chosen to incubate with **PI**, **PC/FBC** or **PI/FBC** nanoparticles for 24 h with or without light irradiated (750 nm, 5 min, 100 $mW \cdot cm^{-2}$). The group without any treatment was acted as the control. Then, the cells medium was replaced with the fresh DMEM containing DCFH-DA solution at the concentration of 10×10^{-6} M at 37 ºC in dark. After treatment for 30 min, the cells were observed *via* confocal laser scanning fluorescence microscope to evaluate the reactive species generation.

The excitation wavelength was 488 nm and the emission wavelength was 525 nm for the DCFH-DA detection.

Cellular Uptake Evaluation

The cellular uptake was determined by using confocal laser scanning microscopy (CLSM). 4T1 cells (2×10^4 cells/well) were seed on glass bottom cell culture dish for 24 h, and then the cells were treated with fresh medium containing **PC/FBC** or **PI/FBC** nanoparticles at the same concentration 20 μg/mL of **FBC** for 24 h. After that, cells were washed carefully and then treated with 4% paraformaldehyde. Then the cells nuclei were stained with Hoechst for 3 min and washed three times with PBS. Finally, intracellular fluorescence of **FBC** was observed by CLSM with excitation at 488 nm and emission at 750 nm.

Cytotoxicity Assay

The cytotoxicity of **PI/FBC** nanoparticles was evaluated by a standard MTT assay. 200 μL of 4T1 cell suspension (1×10^4 cells/mL) was seeded in a 96-well plate and then incubated for 24 h at 37 ℃. Different concentrations of **PC/FBC**, **PI** and **PI/FBC** (**FBC** concentration at 0-8 μg/mL) in fresh DMEM media were added into the wells and *co*-cultured for another 24 h. The cells were washed and irradiated with 750 nm laser (50 mW/cm^2) for 10 min. Before the media was replaced with 200 μL of MTT solution (0.5 mg/mL in DMEM) and cultured for 4 h, the cells were incubated for further 24 h. Finally, 150 μL of DMSO per well was added to replace the MTT solution and dissolve the formazan, and the absorbance value was recorded with a SpectraMax spectrometer at the wavelength of 492 nm. The *in vitro* dark cytotoxicity of nanoparticles was checked by using the same procedure described above but without illumination. In addition, the cytotoxicity of **PC/FBC** nanoparticles at a higher concentration was also determined under a power density of 100 mW/cm^2 (750 nm, 15 min).

Animals and Tumor Xenograft Model

Tumor-bearing BALB/c mice were established and used for *in vivo* imaging: 4T1 cells (10^6 in 200 μL PBS) were subcutaneously injected into the mice, respectively. Once the tumors reached the required volume, the tumor-bearing mice were used for imaging or therapy. The tumor volumes (V) were measured by the length and width of tumors and calculated as $V = 0.5 \times (\text{tumor length}) \times (\text{tumor width})^2$. Relative tumor volume was defined as V/V_0 (V_0 was the tumor volume when the treatment was initiated). All animal studies were conducted on male Balb/c nude mice (four to 5 weeks) in compliance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals approved by the Animal Care and Use Committee of East China University of Science and Technology.

In Vivo Fluorescence Imaging

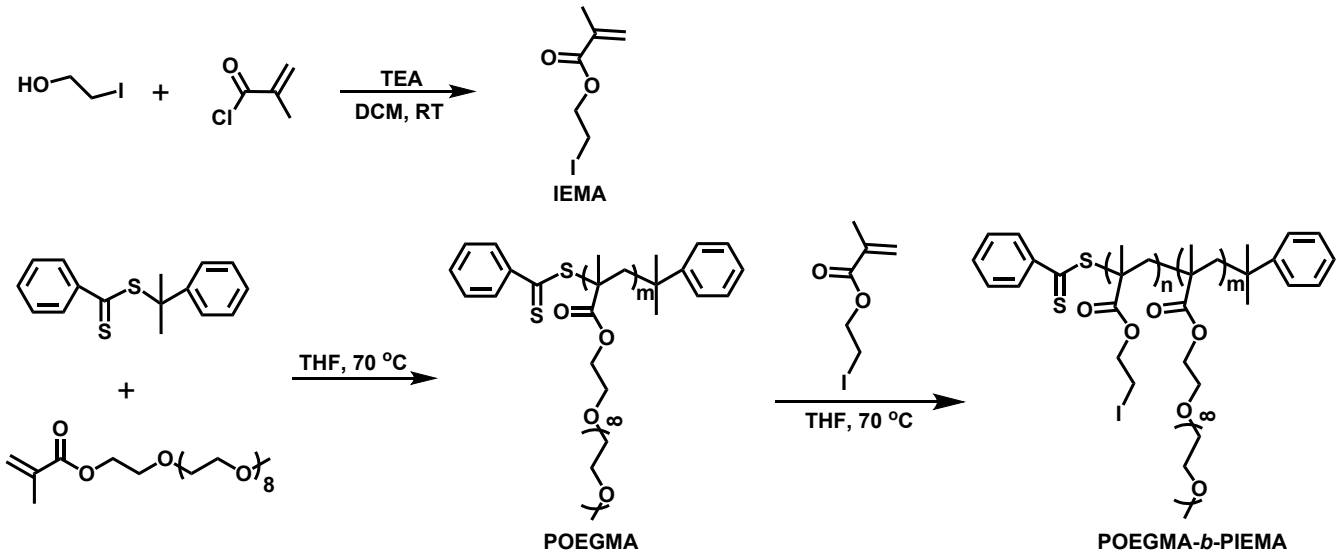
For fluorescence imaging, 200 μL of **PI/FBC** nanoparticles (0.5 mg/mL of **FBC**) were injected into tumor bearing mice through the tail vein. All the image acquisitions were performed with *in vivo* multispectral imaging system (Kodak FX) equipped with excitation bandpass filter at 510 nm and emission at 750 nm when the mice were anesthetized at 0.5, 4, 8, 12, 24 h post-injection.

In Vivo Therapeutical Evaluation of PI/FBC Nanoparticle

The 4T1 tumor-bearing mice were chosen for therapeutical evaluation of the **PI/FBC** nanoparticle. After the tumor volumes of the 4T1 tumor-bearing mice reached about 100 mm^3, the mice were injected with different agents and divided into seven groups (n = 4 for each group): (1) PBS (2) **PI**, (3) **PC/FBC**, (4) **PI/FBC**, (5) **PI+L**, (6) **PC/FBC+L** and (7) **PI/FBC+L** (**FBC** concentration at 0.5 mg/kg). The power density of 750 nm laser was 200 mW/cm^2. The tumor volumes were measured and calculated by vernier caliper every three days. The body weights of mice were recorded during the whole experiments. Then, the mice were sacrificed on the 15^{th} day. The tumors were dissected and weighed. The dissected tumors of the groups (1) PBS (2) **PI**, (3) **PC/FBC**, (4) **PI/FBC**, (5) **PI+L**, (6) **PC/FBC+L** and (7) **PI/FBC+L** were embedded in paraffin and made as 4 μm slices by cryosection. Furthermore, the frozen slices were stained with H&E to further characterize the therapeutic effects. The slices were imaged under an inverted fluorescence microscope.

References

1. X. Han, D. Chen, J. Sun, J. Zhou, D. Li, F. Gong and Y. Shen, *J. Biomater. Sci. Polym. Ed.*, 2016, **27**, 626-642.
2. Z. Liu, T. Cao, Y. Xue, M. Li, M. Wu, J. W. Engle, Q. He, W. Cai, M. Lan and W. Zhang, *Angew. Chem. Int. Ed.*, 2020, **59**, 3711-3717.



Scheme S1. Synthesis of **POEGMA-*b*-PIEMA**.

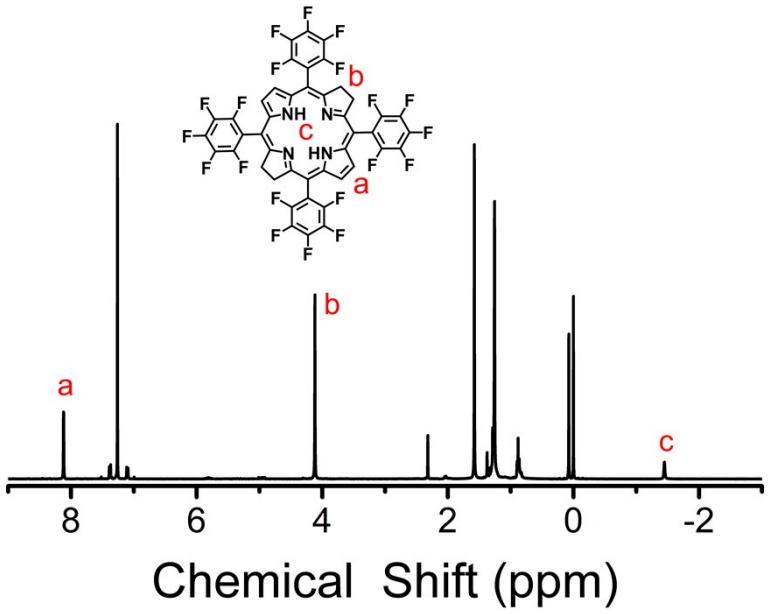


Figure S1. ^{1}H NMR spectrum of **FBC** in $CDCl_3$.

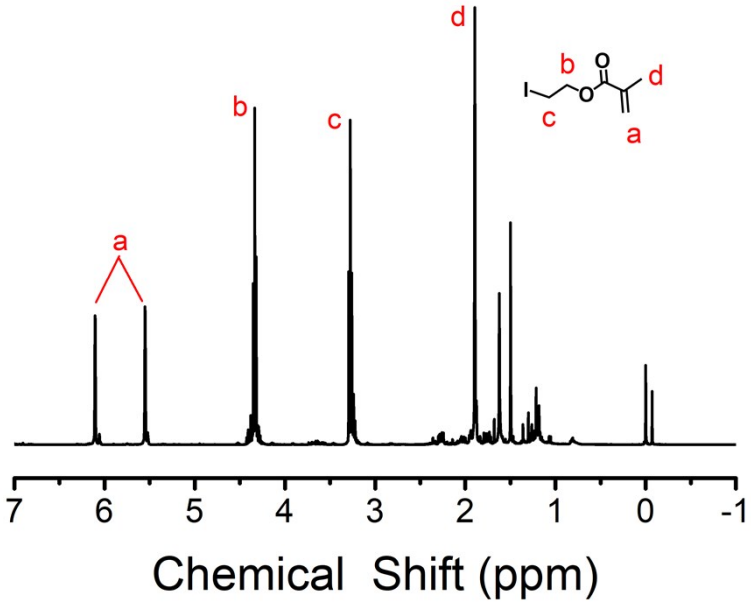


Figure S2. ^{1}H NMR spectrum of IEMA in $CDCl_3$.

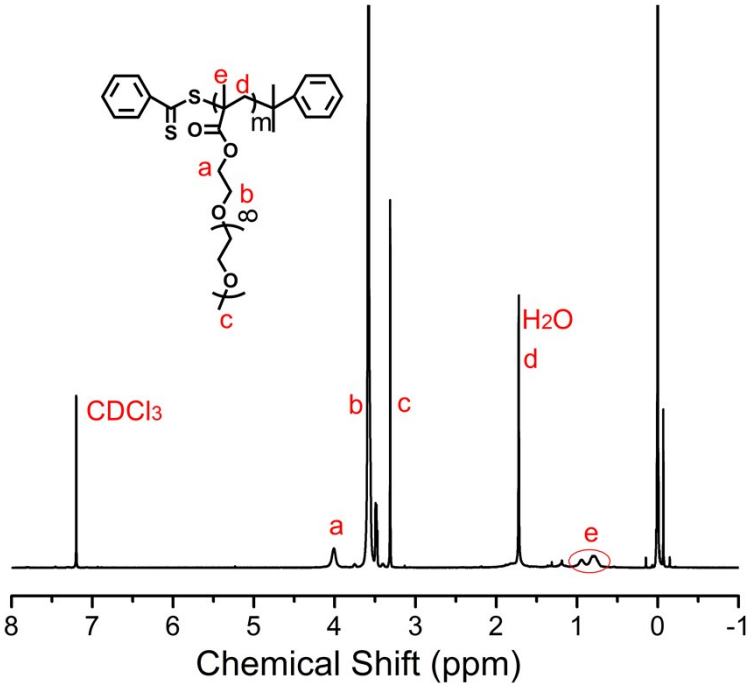


Figure S3. ^{1}H NMR spectrum of POEGMA homopolymer in $CDCl_3$.

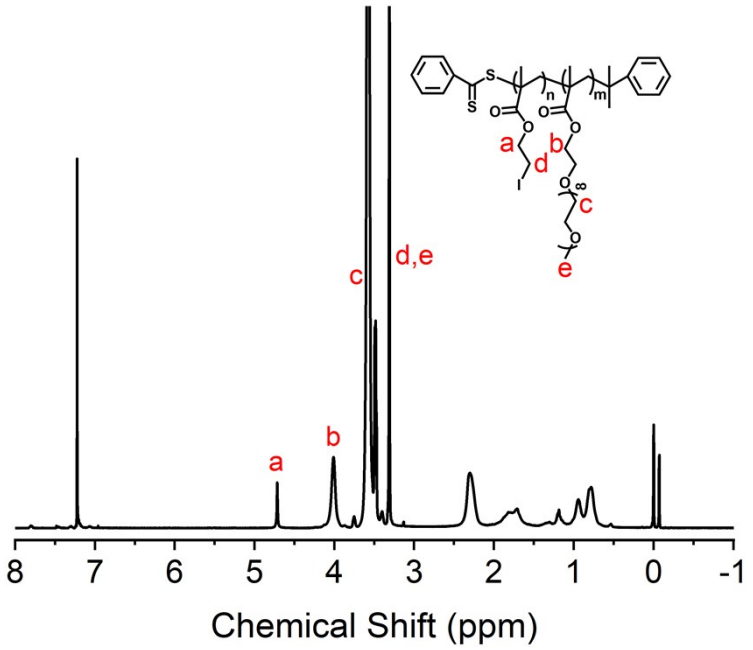


Figure S4. ^{1}H NMR spectrum of POEGMA-*b*-PIEMA block copolymer in $CDCl_3$.

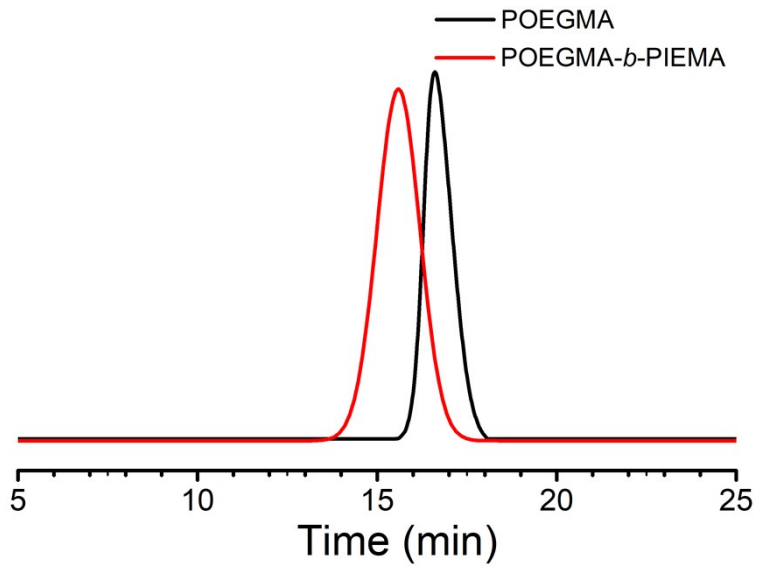


Figure S5. GPC traces of POEGMA and POEGMA-*b*-PIEMA.

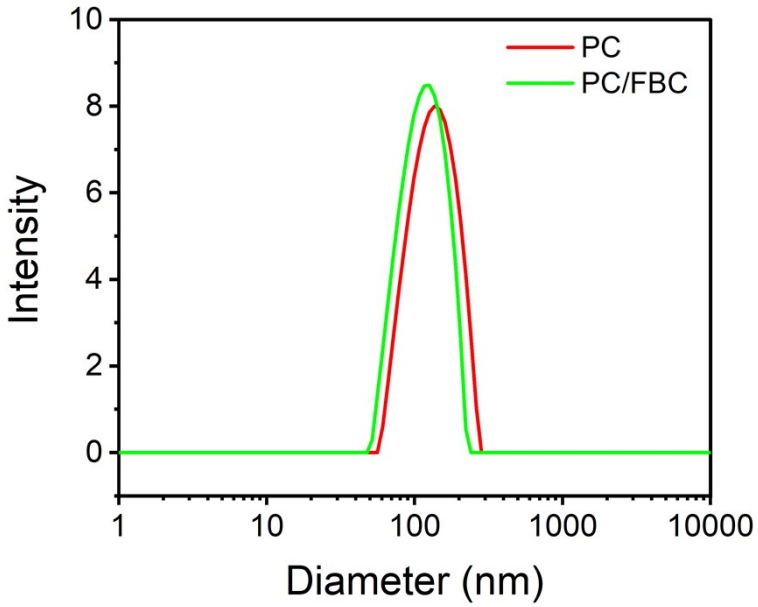


Figure S6. Size distribution of nanoparticles determined by dynamic light scattering.

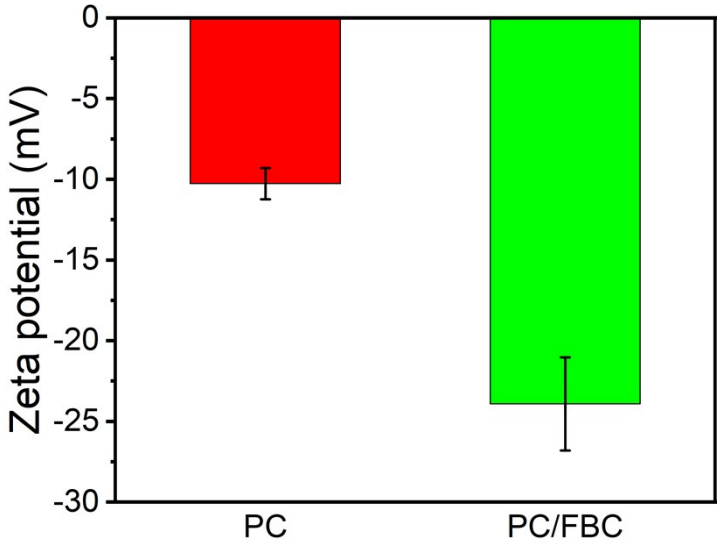


Figure S7. Zeta potential of PC and **PC/FBC** nanoparticles.

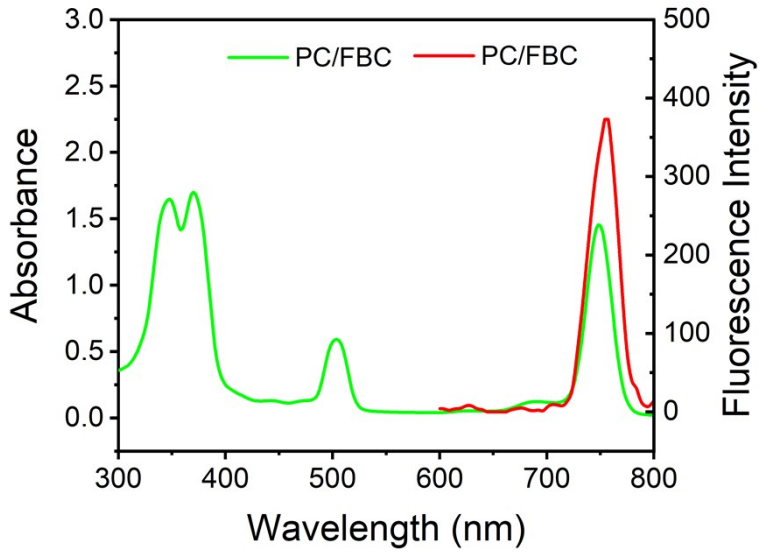


Figure S8. UV-vis absorption and fluorescence spectra of **PC/FBC** nanoparticles in PBS (pH = 7.4, EX = 510 nm).

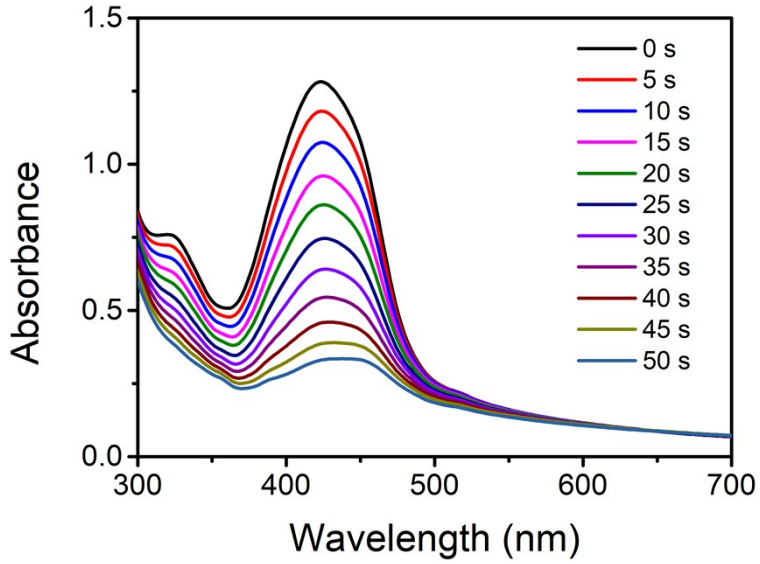


Figure S9. UV-vis absorption spectra of DPBF incubated with **PC/FBC** nanoparticles under 750 nm laser irradiation (PBS, pH = 7.4, 50 mW/cm^2).

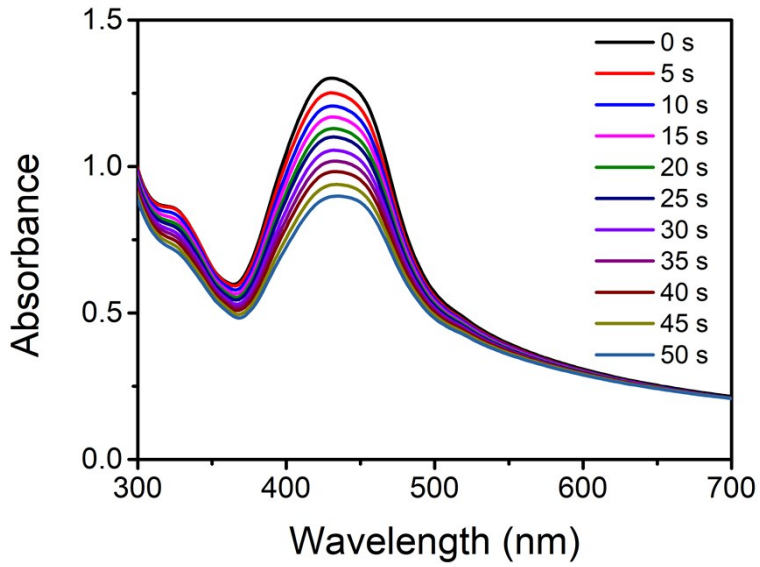


Figure S10. UV-vis absorption spectra of DPBF incubated with **PI/FBC** nanoparticles under 750 nm laser irradiation (PBS, pH = 7.4, 50 mW/cm^2).

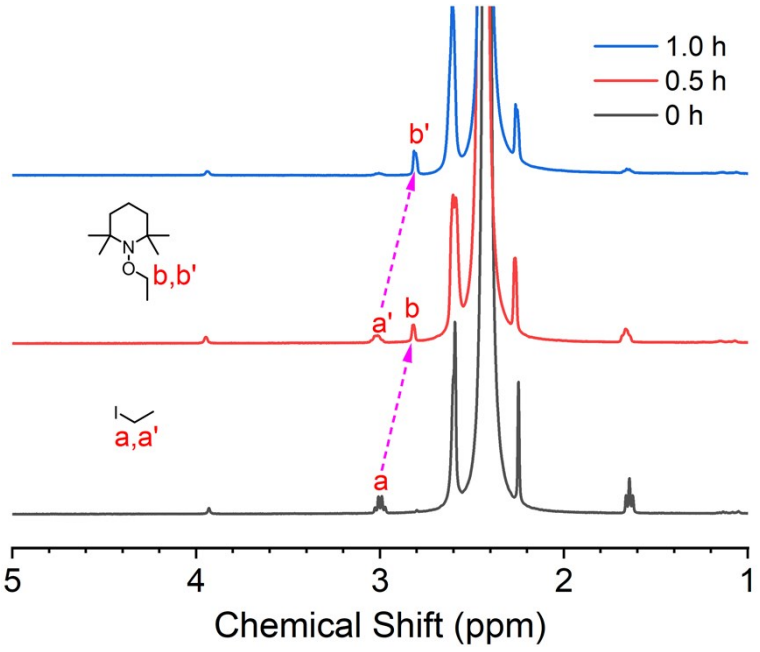


Figure S11. ^{1}H NMR spectra of TEMPO in $CDCl_3$ with different light irradiation time (750 nm).

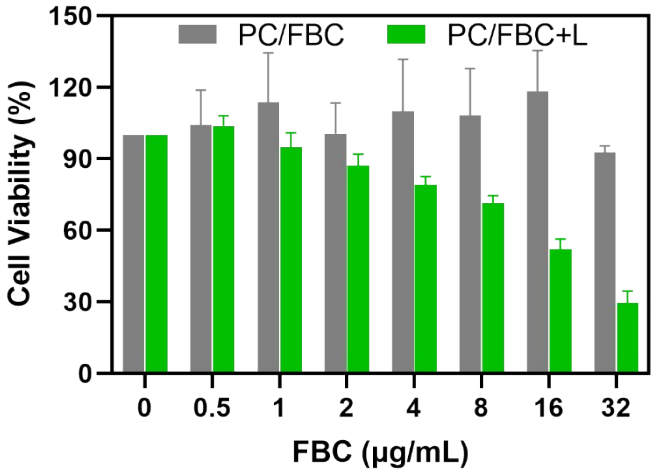


Figure S12. Relative viabilities of 4T1 cells incubated with **PC/FBC** nanoparticles (750 nm, 100 mW/cm^2, 15 min).

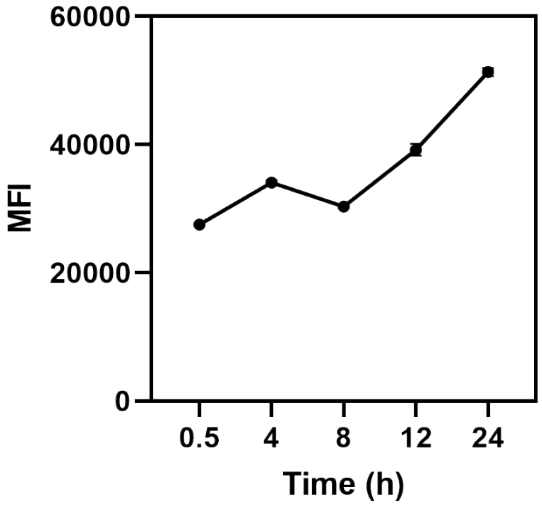


Figure S13. Quantitative fluorescence intensity of tumors after intravenous injection of **PI/FBC**.

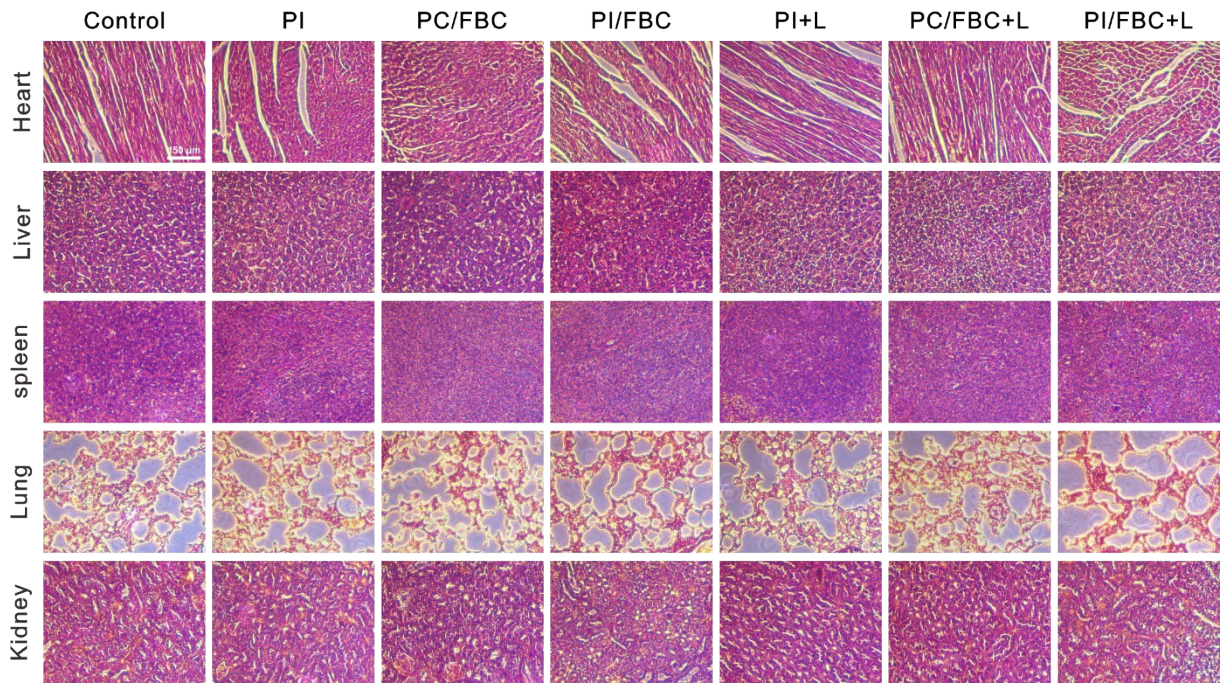


Figure S14. Histological data (hematoxylin and eosin-stained images) obtained from the liver, spleen, kidney, heart, and lung (scale bar: 150 μm).