Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.

Supporting Information

Photo-induced Specific Intracellular Release EGFR Inhibitor from Enzyme/ROS-dual Sensitive Nano-platforms for Molecular Targeted-Photodynamic Combinational Therapy of Non-small Cell Lung Cancer

Man Zhang [a], Xuequan Zhang [b], Shengsheng Cai [b], Heng Mei [b], Yanmei He [b], Dennis Huang [c], Wenqiang Shi [b], Sai Li [a,], Jun Cao [b,]*, Bin He [b]*

[a] *School of Chemical Engineering, Sichuan University, Chengdu 610065 China*

[b] *National Engineering Research Center for Biomaterials, Sichuan University, Chengdu 610064 China*

[c] *Departments of Biomedical Engineering, University of Texas at Austin, Austin 78712, USA*

** E-mail: lisai@scu.edu.cn (S. L), caojun@scu.edu.cn (J. C); Fax: +86-28-85412923; Tel: +86-28-85412923*

1. MATERIALS AND METHODS

1.1 Materials. 7-Ethyl-10-hydroxycamptothecin (SN38) was available from HEOWNS Co. Ltd (Tianjin, China). Trifluoroacetic acid (TFA) and triethylamine (TEA) were purchased from Asta Tech Biopharm Co. Ltd (Chengdu, China). Ammonia, tween 80, disodium phosphate, pyridine and potassium dihydrogen phosphate were purchased from Kelong Chemical Co. (Chengdu, China).

1.2 Synthesis of methoxy poly (ethylene glycol)-poly(L-lysine) (mPEG-PLL). The synthesis of the methoxy poly (ethylene glycol)-poly(L-lysine) (mPEG-PLL) was referred by our previous study [1-2].

Briefly, pyridine (0.95 g, 12 mmol) and TsCl (2.86 g, 15 mmol) in 30 mL CH_2Cl_2 were added dropwise into mPEG (7.5 g, 3 mmol) in anhydrous CH_2Cl_2 and stirred in an ice-water bath for several hours. After stirring at room temperature for 4 days under nitrogen atmosphere, the solution was filtrated, concentrated and precipitated using excess cold diethyl ether, followed by recrystallized in ethanol, collected and dried in vacuum. The purified product was added into 25 wt% ammonia water solution under vigorously stirring at room temperature for 3 days. The solution was extracted with CH_2Cl_2 for four times and then dried with anhydrous magnesium sulfate. After removed the solvent by rotary evaporator, mPEG-NH_2 was obtained through recrystallized in ethanol twice and dried in vacuum.

Triphosgene (6.88 g, 0.023 mol) in THF was added dropwise into N_6-Cbz-L-Lysine (5 g, 0.018 mol) in anhydrous THF under nitrogen atmosphere in an oil bath at 50 °C. The reaction was stopped when the solution became clear. The clear solution was condensed using rotary evaporator, precipitated in large amounts of anhydrous hexane for three times. The acquired product (NCA) was dried in vacuum.

Then, NCA (5 g, 0.016 mol) and mPEG-NH_2 (0.82 g, 0.4 mmol) were mixed in CH_2Cl_2 and stirred at room temperature under nitrogen atmosphere for 3 days. Afterwards, the mixture solution was precipitated in anhydrous diethyl ether and collected through filtration to obtain mPEG-PLLBz. mPEG-PLLBz was dissolved in trifluoroacetic acid, and then hydrogen bromide was added dropwise for stirring 1 h room temperature until the solution became clear. Subsequently, the mixture was washed by a large of cold anhydrous diethyl ether for three times. After dialyzed with deionized water, the copolymer (mPEG-PLL) was obtained by freeze-drying.

1.3 Synthesis of mPEG-PLL conjugated Ce6 with thioether linker. mPEG-PLL (435 mg, 0.075 mmol) was dissolved in 10 mL of anhydrous DMSO by stirring for 2 h at room temperature under nitrogen atmosphere. Subsequently, the GMA (43 mg, 0.3 mmol) dissolved in anhydrous DMSO was added dropwise into the mPEG-PLL solution at 0 °C for stirring 4 h and dialyzed against DMSO for several days. After being dialyzed against deionized water consecutively to remove DMSO, mPEG-PLL-GMA copolymer was obtained by freeze-drying.

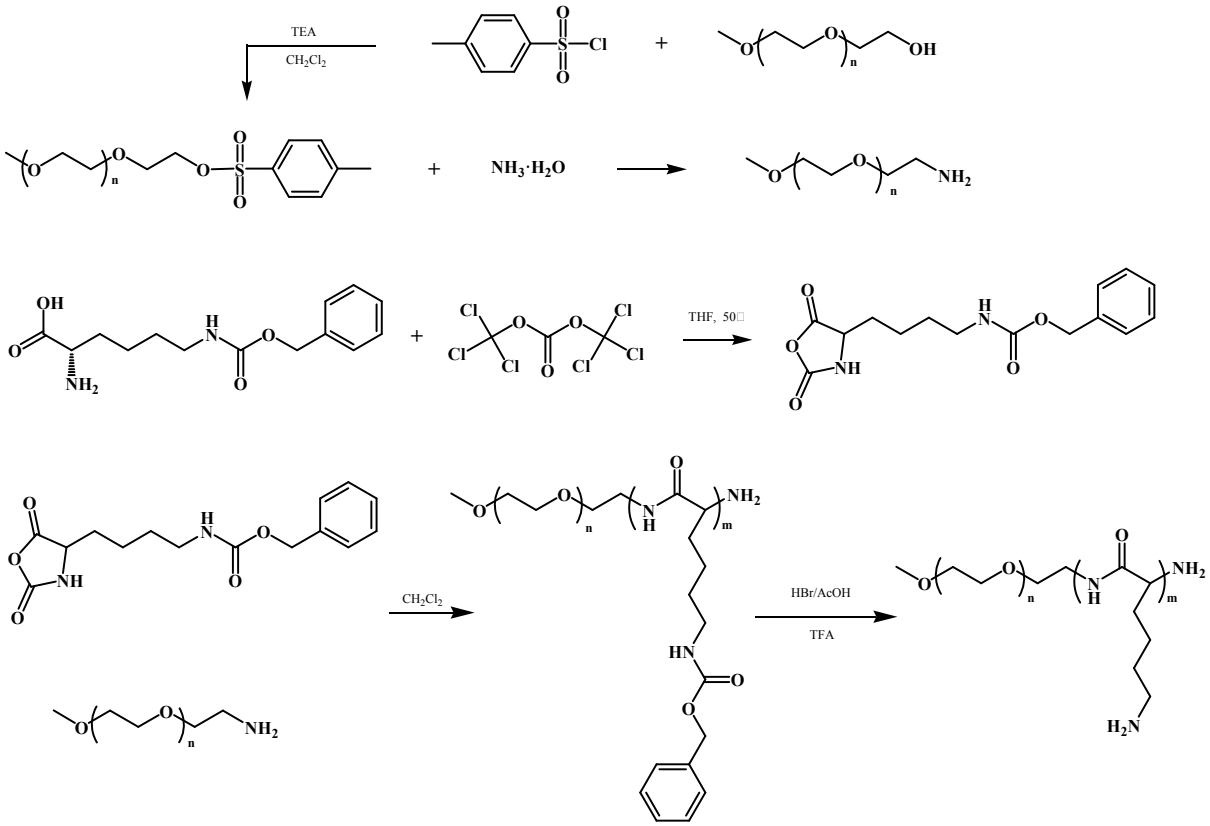
Thioether linker-conjugated Ce6 (Ce6-TL) was synthesized through thiol-ene click reaction as previously described[3]. Briefly, Ce6 (180 mg, 0.3 mmol) was dissolved in DMSO, and the solution was bubbling with nitrogen for 1 h to exhausted oxygen. Then the solution was added dropwise into DMSO solution of bis(2-mercaptoethyl) sulfide (46 mg, 0.3 mmol) and DMPA (76 mg, 0.3 mmol) under 365 nm UV irradiation for stirring 4 h. Then, the solution was added into DMSO solution of mPEG-PLL-GMA copolymer under 365 nm UV irradiation for 4 h. The reaction solution was dialyzed against DMSO and deionized water consecutively to remove the unreacted monomers and the organic solvent for 3 days. The amphiphilic copolymer, mPEG-PLL conjugated Ce6 with thioether linker (mPEG-PLL-g-R-Ce6), was acquired by freeze-drying. The structure of mPEG-PLL-g-R-Ce6 was characterized by the ^{1}HNMR spectra

spectrum (AV-400, Bruker, America) using DMSO-d_6 as the solvent, and tetramethylsilane was used as the internal standard.

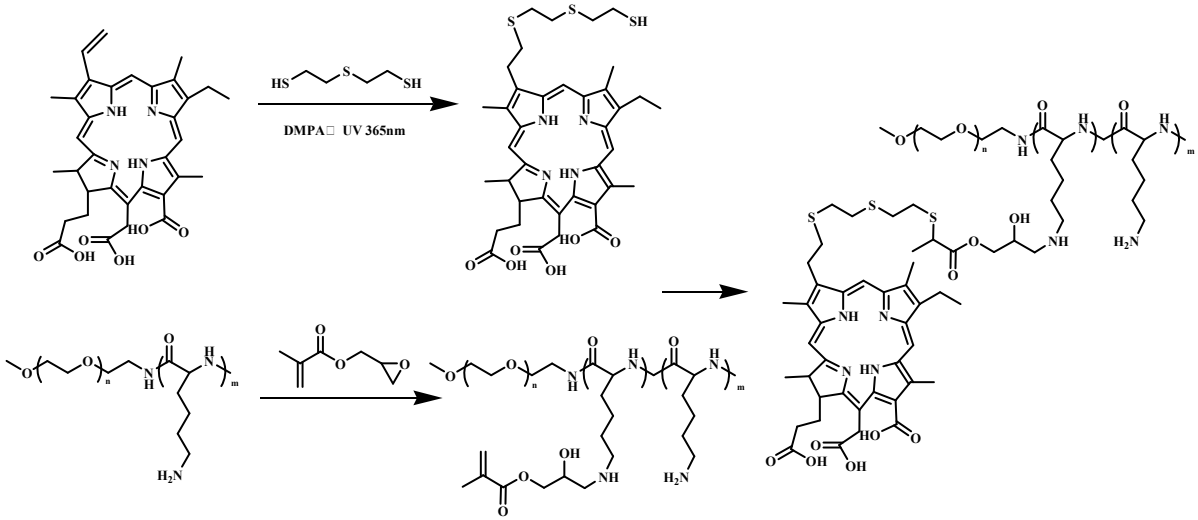
1.4 The GPC characterization of mPEG-PLL-g-R-Ce6. The molecular weight and molecular weight distribution of mPEG-PLL-g-R-Ce6 polymer were detected by gel permeation chromatography (GPC, Agilent GPC PL50) based on the standard of polystyrene (THF, 0.6 mL/min flow rate).

1.5 The Oxidation of mPEG-PLL-g-R-Ce6 in ROS conditions. The ROS-responsive property of mPEG-PLL-g-R-Ce6 polymer was investigated by ^{1}HNMR. Firstly, the fresh aqueous solution of 10 mM H_2O_2 was prepared to produce ROS *in situ*. Afterwards, the H_2O_2 solution was added into the DMSO solution of mPEG-PLL-g-R-Ce6 (10 mg) and the mixture was incubated at 37 °C for 24 h. Then the solution was dialyzed, lyophilized and characterized by ^{1}HNMR. The size change of the PGBCA in response to ROS conditions was measured by DLS. In brief, the fresh aqueous solution of 1 mM H_2O_2 was added into 1 mL of PGBC micellar solution (1 mg/mL). Then the solution was incubated at 37 °C for 24 h and the size change was measured by DLS.

1.7. Cell culture. Adenocarcinomic human alveolar basal epithelial cell line (A549) and mouse fibroblast cell line (3T3) were used for *in vitro* evaluation. These cell lines were all cultured at 37 °C in RPMI 1640 medium or DMEM medium containing 10% FBS and 1% penicillin/streptomycin in an incubator with 5% CO_2.



Scheme S1. The synthetic routes of mPEG-PLL copolymer.



Scheme S2. The synthetic routes of mPEG-PLL-g-R-Ce6 copolymer.

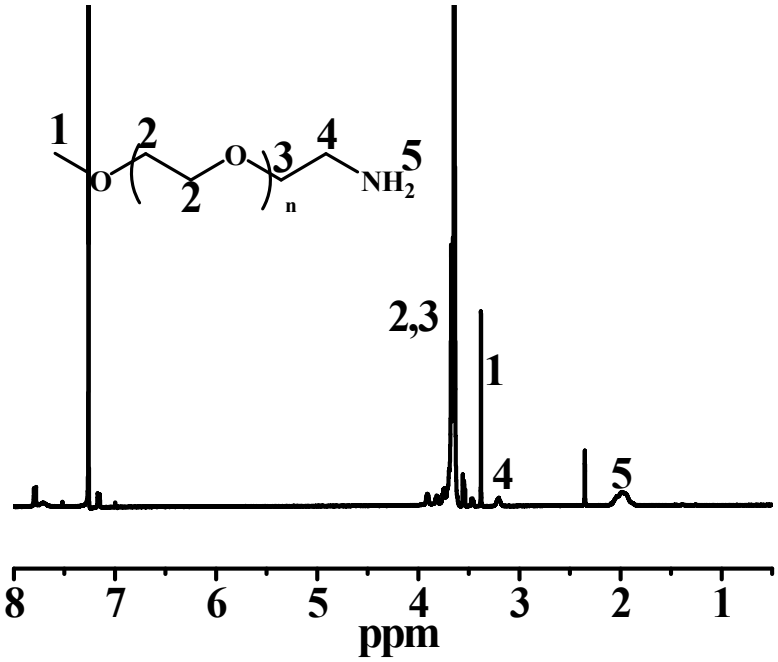


Figure S1. The ^{1}HNMR spectra of mPEG-NH_2.

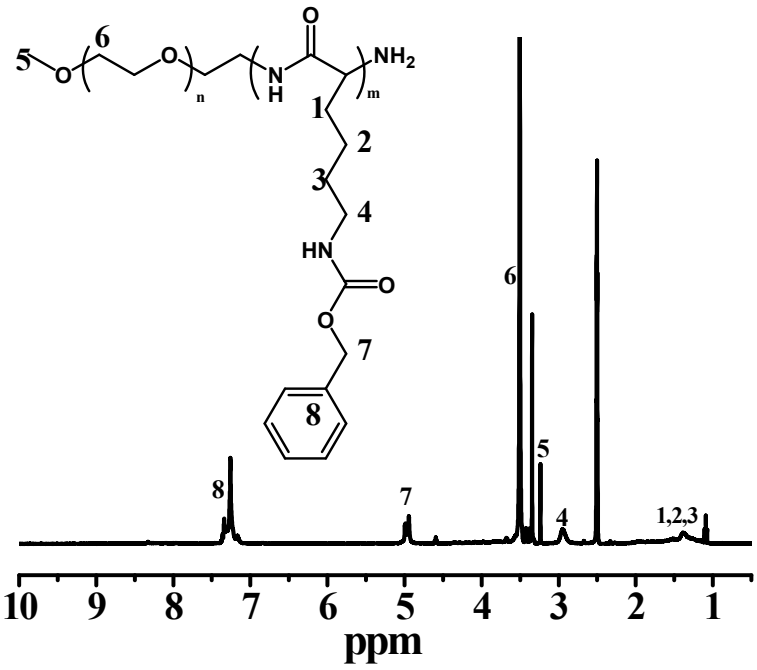


Figure S2. The ^{1}HNMR spectra of mPEG-PLLBz.

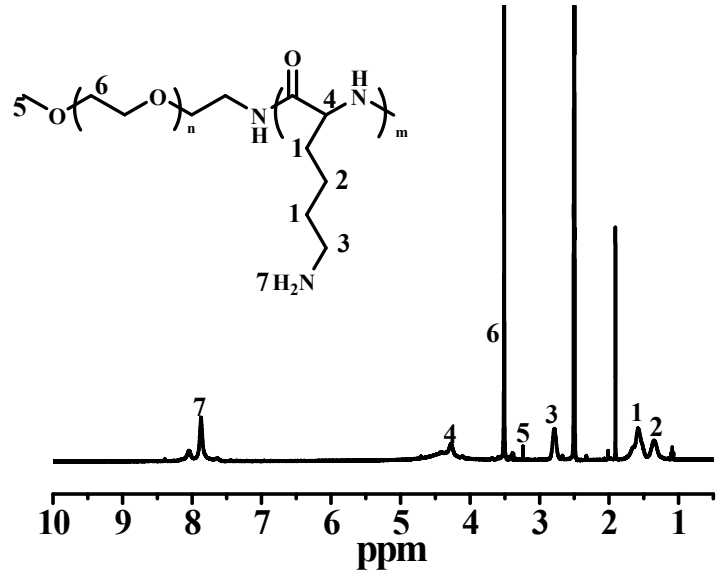


Figure S3. The [1]HNMR spectra of mPEG-PLL.

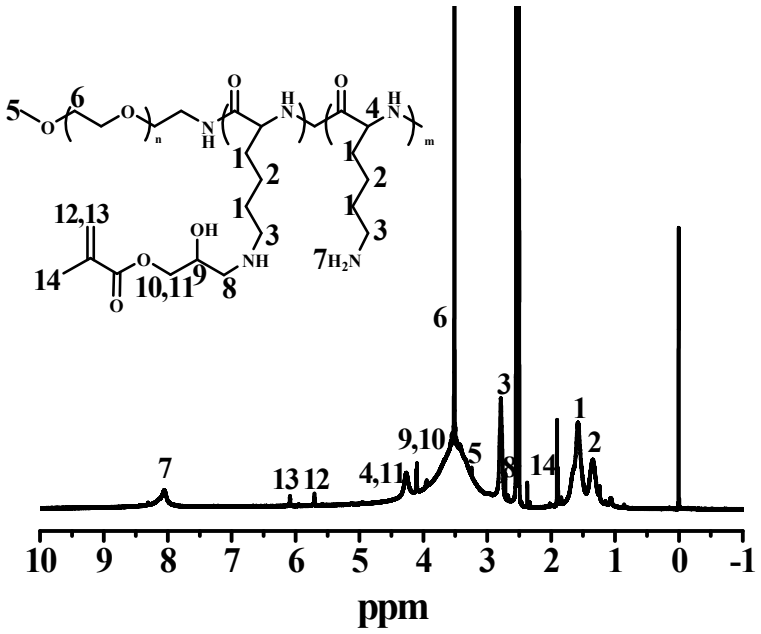


Figure S4. The [1]HNMR spectra of mPEG-PLL-GMA.

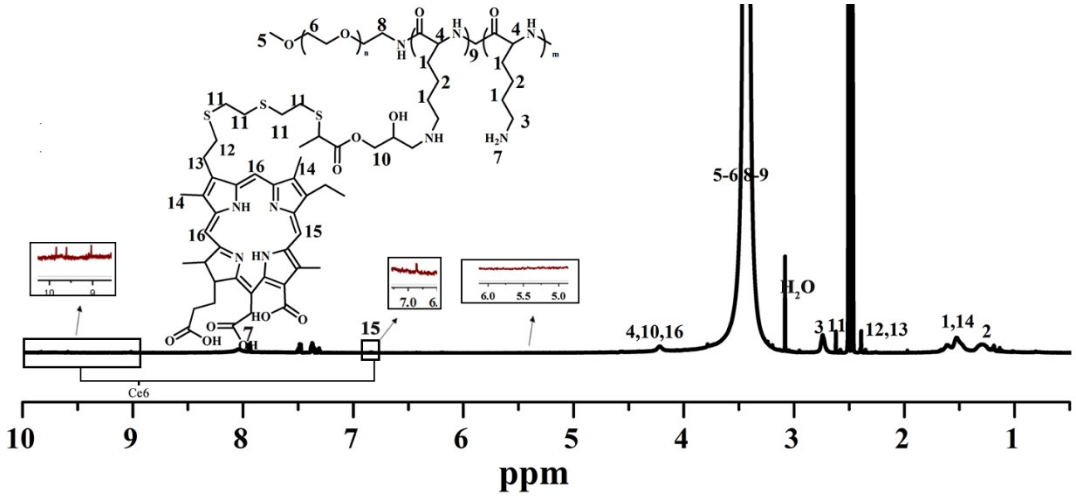


Figure S5. [1]HNMR spectra of mPEG-PLL-g-R-Ce6 copolymers.

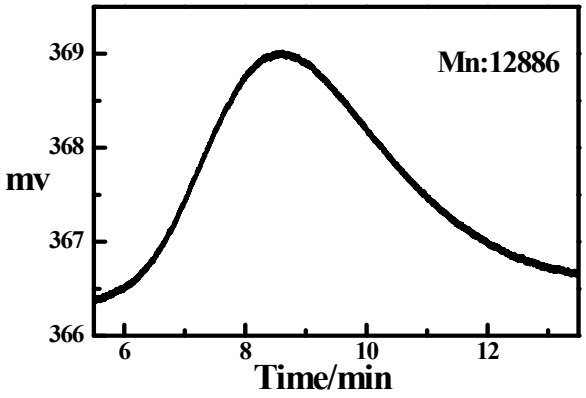


Figure S6. The GPC curve of mPEG-PLL-g-R-Ce6.

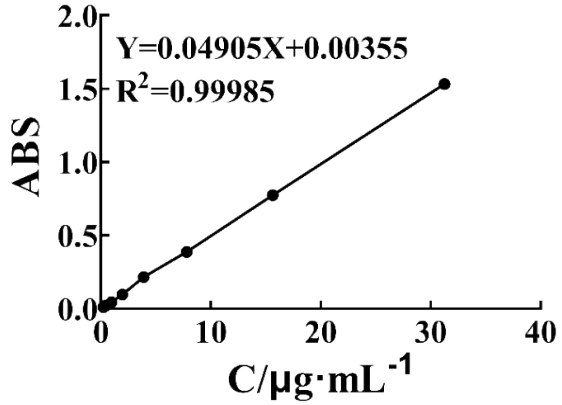


Figure S7. The standard curve of free AFT using ultraviolet spectrophotometry measured by HPLC.

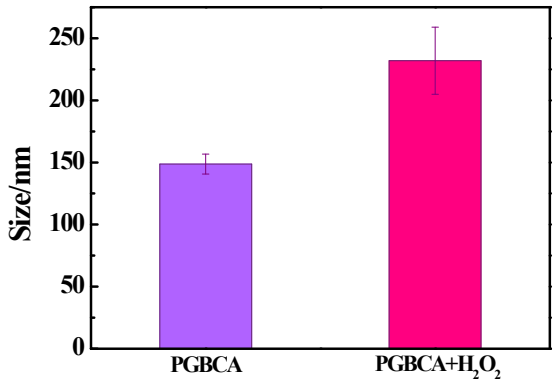


Figure S8. The size change of PGBCA incubated with H_2O_2.

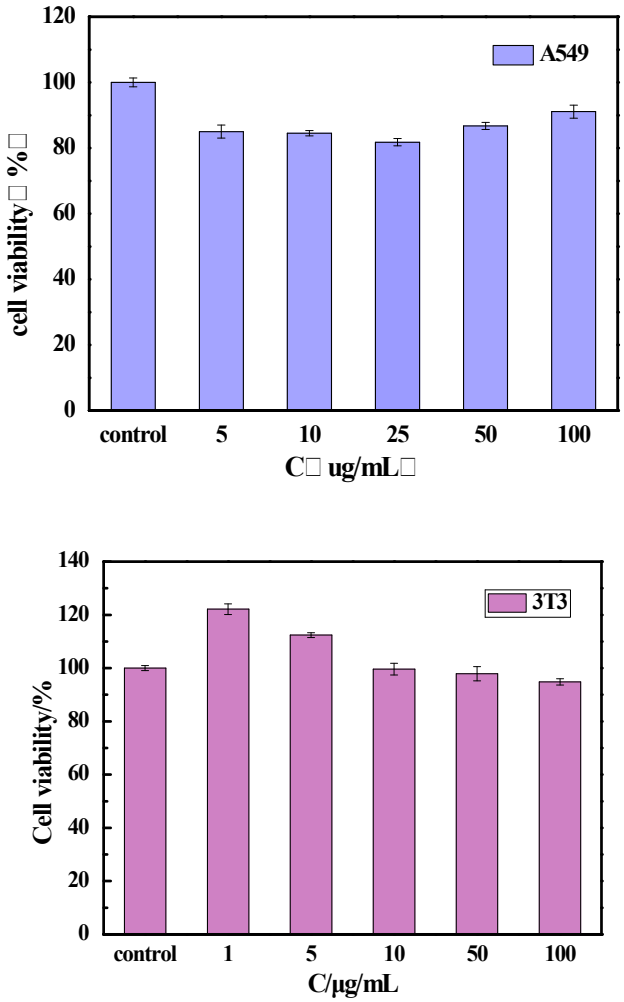


Figure S9. The cell viability of A549 cells and 3T3 cells incubated with blank nanoparticles.

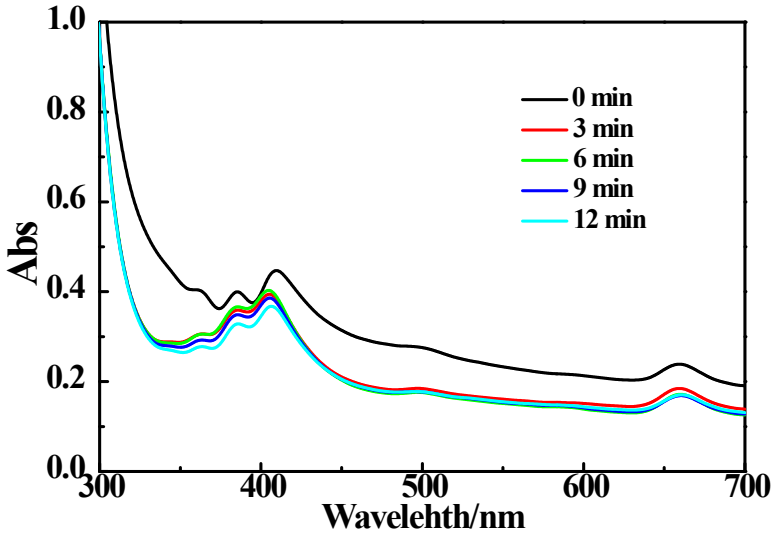


Figure S10. The UV absorption of HPGBC loaded with DPBF after NIR irradiation at different time.

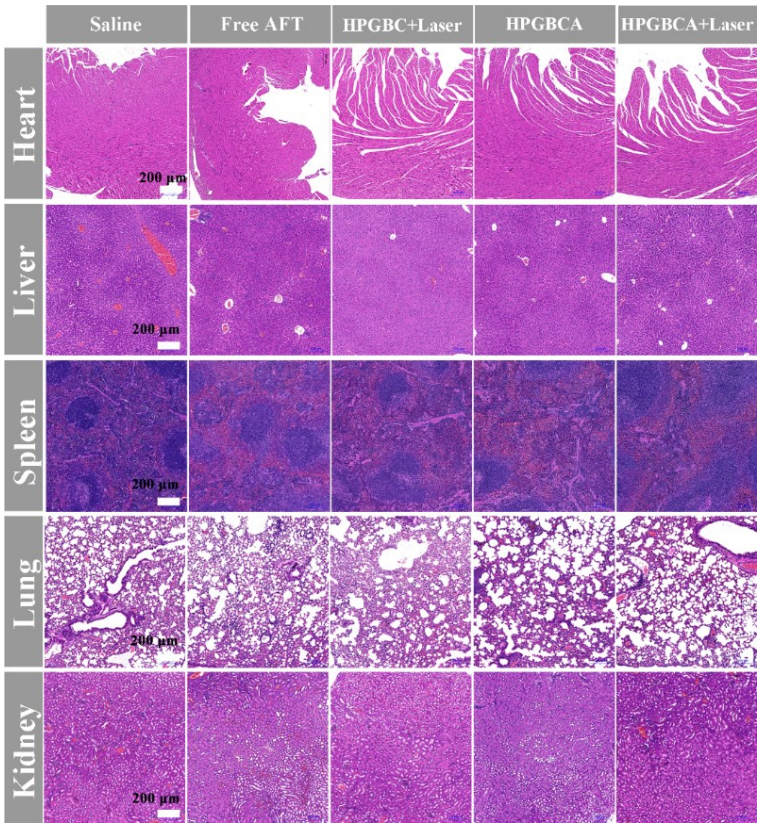


Figure S11. H&E staining images of main organs separated from tumor-bearing mice treated with different formulations.

References

1. Cao, J.; Su, T.; Zhang, L.; Liu, R.; Wang, G.; He, B.; Gu, Z., Polymeric micelles with citraconic amide as pH-sensitive bond in backbone for anticancer drug delivery. *International journal of pharmaceutics* **2014,** *471* (1-2), 28-36.
2. Xu, T.-T.; Li, J.-H.; Cheng, F.-R.; Zhang, Y.-X.; Cao, J.; Gao, W.-X.; He, B., Fabrication of a polypseudorotaxane nanoparticle with synergistic photodynamic and chemotherapy. *Chinese Chemical Letters* **2017,** *28* (9), 1885-1888.
3. Bharathiraja, S.; Moorthy, M. S.; Manivasagan, P.; Seo, H.; Lee, K. D.; Oh, J., Chlorin e6 conjugated silica nanoparticles for targeted and effective photodynamic therapy. *Photodiagnosis and photodynamic therapy* **2017,** *19*, 212-220.