Supporting Information

Porphyrin-Based Drug-Delivery Nanoparticles for Photodynamic Therapy and Photoactivated Cascade Chemotherapy

Lihua Du[a], Kejing Zeng[b], Huaping Huang[a], Haozhe He[a,*]

[a] Digestive Diseases Center, The Seventh Affiliated Hospital, Sun Yat-Sen University, Shenzhen 518107, China.

[b] Department of Endocrinology, Department of diabetes and obesity reversal research center, Guangdong Second Provincial General Hospital, Guangzhou 510317, China.

* Corresponding author: hehzh8@mail.sysu.edu.cn (H. He).

Materials and methods

Materials and measurements

All chemicals and solvents were obtained from commercial suppliers and utilized as received unless otherwise specified. Dynamic light scattering (DLS) analyses were conducted using an Elite Sizer instrument. A JEOL JEM-F200 spherical aberration corrected transmission electron microscope was employed to acquire transmission electron microscopy (TEM) images. Absorption spectra were recorded on a PerkinElmer UV750 ultraviolet-visible (UV-vis) spectrometer. X-ray photoelectron spectroscopy (XPS) data were collected with a VG Scientific ESCALAB 250 system. Gas sorption isotherms were measured on a Micromeritics ASAP2020M analyzer. Confocal laser scanning microscopy (CLSM) imaging was performed with a Leica SP8 system. For in vivo and ex vivo fluorescence imaging, a Carestream IS 4000 imaging system (USA) was used.

Synthesis of the $THPP_{TK}$-PEG-FA NPs

The thioketal (TK) linker was prepared following a reported procedure. $THPP_{TK}$ was synthesized by esterifying THPP with TK. Specifically, THPP (0.5 mmol), TK (2 mmol), EDC (4 mmol), and DMAP (4 mmol) were dissolved in tetrahydrofuran (THF, 50 mL). The reaction mixture was stirred at 50°C in the dark for 48 h. Subsequently, THF was evaporated using a rotary evaporator. The crude product was washed repeatedly with methanol and ice-cold diethyl ether until the supernatant became colorless, removing unreacted THPP and TK. Next, $THPP_{TK}$ was conjugated with PEG via an esterification reaction. $THPP_{TK}$ (0.5 g), FA-PEG-COOH (2.5 g), EDC (0.5 g),

and DMAP (0.5 g) were dissolved in THF (50 mL) and stirred at 50°C in the dark for 24 h. After rotary evaporation of THF, the mixture was washed with methanol and ice-cold diethyl ether. The product was then dialyzed (MWCO 3500 Da) against distilled water for 24 h, with water changed every 6 h. Finally, $THPP_{TK}$-PEG-FA NPs were obtained as a powder after freeze-drying.

Preparation of DOX-loaded and Cy-loaded NPs

DOX-loaded nanoparticles were prepared using a simple method. Briefly, DOX (30 mg) was dissolved in DMF (1 mL) and stirred for 30 minutes. $THPP_{TK}$-PEG-FA NPs (100 mg) were added to this solution, and stirring continued for another 2 h. The mixture was then slowly introduced into 19 mL of water under magnetic stirring. After 2 h of additional stirring, the solution was transferred to a dialysis bag (MWCO 3500 Da) and dialyzed for 24 h to remove free DOX and impurities, with water replenished every 6 h. The contents of the dialysis bag were freeze-dried to obtain DOX@$THPP_{TK}$-PEG-FA NPs. To determine drug loading content (DLC) and drug loading efficiency (DLE), the NPs were dissolved in deionized water. The DOX concentration was quantified by measuring UV absorbance at 480 nm using a standard calibration curve. DLC and DLE were calculated as follows:

DLC (wt%) = (weight of loaded drug / weight of drug-loaded micelles) × 100%

DLE (wt%) = (weight of loaded drug / weight of drug in feed) × 100%

For Cy-loaded NPs, the procedure was similar except that Cy (20 mg) was dispersed in DMF (1 mL). The THPP content was measured analogously to the DOX content.

Evaluation of the nanoparticles stability

$THPP_{TK}$-PEG-FA and DOX@$THPP_{TK}$-PEG-FA NPs were dispersed in PBS (pH 7.4, 0.01 M). Aliquots (3 mL) were taken at designated time points and analyzed by DLS to assess stability.

Detection of Extracellular $^1O_2$ by SOSG

To detect singlet oxygen generation, $THPP_{TK}$-PEG-FA (20 μg/mL) and SOSG (10 μM) were exposed to a 660 nm laser (50 mW cm^{-2}) for different durations. The fluorescence of oxidized SOSG at 525 nm (excitation 494 nm) was monitored over time using an F-4500 fluorescence spectrophotometer. The same experiment was conducted with DOX@$THPP_{TK}$-PEG-FA. Error bars represent standard deviations from triplicate measurements.

***In vitro* DOX release**

Freeze-dried DOX-loaded NPs were dissolved in PBS (pH 7.4) at 1 mg mL^{-1}. The solution was placed in a dialysis bag (MWCO 3500 Da) and immersed in 20 mL of buffer at the same pH. The system was maintained at 37°C in a constant temperature shaker. At predetermined intervals, 3 mL of the external buffer was sampled for UV-vis analysis and replaced with fresh buffer. The amount of released DOX was determined from the absorbance at 480 nm using a DOX calibration curve. Cumulative DOX release was calculated as a function of incubation time.

Cell lines

K562 leukemia cells were cultured in specialized K562 medium (Wuhan Pricella Biotechnology Co., Ltd.) at 37°C in a humidified 5% CO_2 atmosphere.

Cytotoxicity

Cytotoxicity was assessed by MTT assay. Sample solutions were diluted with K562 medium to desired concentrations. K562 cells were seeded in 96-well plates at 10^4 cells per well and incubated for 24 h. Various concentrations of samples were added, with three replicate wells per concentration. After 12 h of co-incubation, some wells were irradiated with a 660 nm laser (50 mW cm^{-2}) for 10 min, followed by another 12 h of incubation. Then, MTT solution (20 μL, 5 mg/mL in PBS) was added to each well, and plates were incubated for 4 h at 37°C. The medium was removed, and formazan crystals were dissolved in DMSO (150 μL/well). After shaking for 5 min, absorbance at 492 nm was measured using a BioTek Synergy2 Gen5 microplate reader.

In vitro cellular uptake

Cellular uptake was evaluated by CLSM. K562 cells were seeded in 6-well plates containing sterile coverslips at 1×10^5 cells/well and cultured for 24 h. The medium was replaced with fresh medium containing $THPP_{TK}$-PEG-FA or DOX@$THPP_{TK}$-PEG-FA NPs (20 μg/mL, equivalent THPP concentration). After 2 h of incubation at 37°C, some cells were irradiated with a 660 nm laser (50 mW cm^{-2}) for 10 min and incubated for another 2 h to assess PDT effects on uptake. Cells were then washed with ice-cold PBS, fixed with 4% formaldehyde, and nuclei were stained with DAPI. CLSM images were captured under consistent settings.

Detection of intracellular ROS generation

Intracellular ROS was detected using a DCFH-DA as a fluorescence probe. K562 cells were seeded on coverslips in 6-well plates at 1×10^5 cells/well and allowed to adhere for 24 h. Cells were treated with NPs (20 μg/mL, equivalent THPP concentration) for

2 h. Some groups were then irradiated (660 nm, 50 mW cm^{-2}, 10 min) and incubated for an additional 2 h. CLSM images were acquired under standardized conditions.

Animals and tumor model

Female Balb/c nude mice (4-6 weeks old) were supplied by the Guangdong Medical Laboratory Animal Center. All animal procedures were approved by the Institutional Animal Care and Use Committee of Sun Yat-sen University (Guangzhou, China) and complied with national regulations. A tumor model was established by subcutaneously injecting K562 cells (2×10^6) into the backs of mice.

Animal experiments and biodistribution analysis

Mice bearing K562 tumors (~100 mm^3) were randomly divided into five groups. All groups received a tail vein injection of 100 μL of PBS, $THPP_{TK}$-PEG-FA, or DOX@$THPP_{TK}$-PEG-FA (THPP dose: 5 mg kg^{-1}). To determine the optimal irradiation time, mice were injected with Cy@$THPP_{TK}$-PEG-FA (200 μg mL^{-1}, 100 μL) and imaged at 8, 12, and 24 h post-injection. At 24 h, mice were euthanized, and major organs (heart, liver, spleen, lung, kidneys) and tumors were collected for imaging. For therapeutic groups requiring laser treatment, tumors were irradiated with a 660 nm laser (200 mW cm^{-2}) for 10 min.

H&E, TUNEL and Ki-67 staining

Tissue samples were processed following standard histological protocols. Tissues were paraffin-embedded, sectioned, and mounted on slides. After H&E, TUNEL, and Ki-67 staining, images were captured using a Leica DM28 inverted fluorescence microscope (Germany).

Statistical analysis

Data are presented as mean ± standard deviation (SD). Group comparisons were performed using one-way ANOVA with Tukey's post-hoc test. Significance levels are indicated as *P < 0.05, **P < 0.01, and ***P < 0.001.

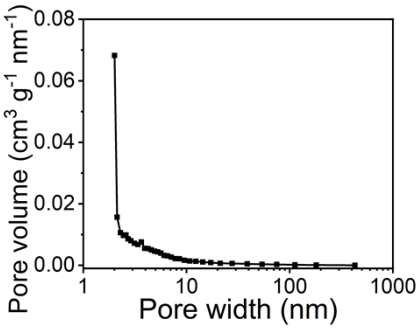

Figure S1. The corresponding pore size distribution profile derived from the isotherms.

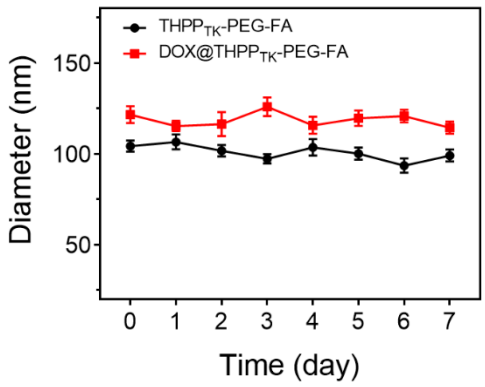

Figure S2. Stability test of THPP$_{TK}$-PEG-FA NPs and DOX@THPP$_{TK}$-PEG-FA NPs dissolved in PBS (pH 7.4) for different time periods determined by DLS.

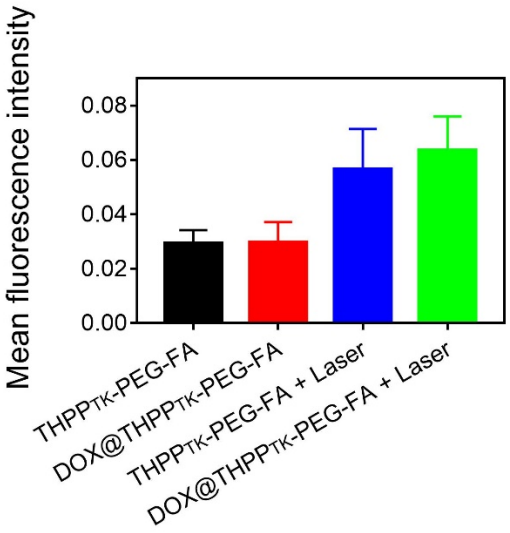

Figure S3. Mean fluorescence intensity of laser-induced ROS generation in K562 cells treated with $THPP_{TK}$-PEG-FA or DOX@$THPP_{TK}$-PEG-FA NPs.

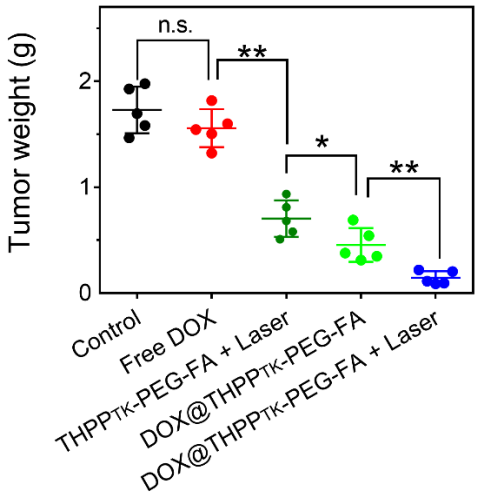

Figure S4. Quantitative analysis of tumor weight on day 18.

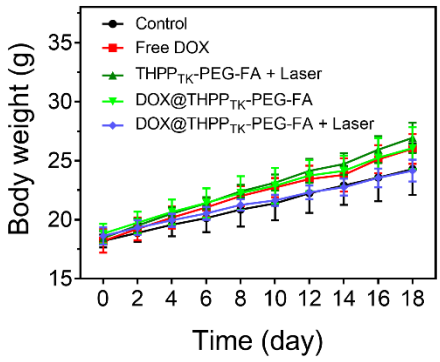

Figure S5. Body weight of the K562 cancer bearing mice of different groups after treatments (n = 5).

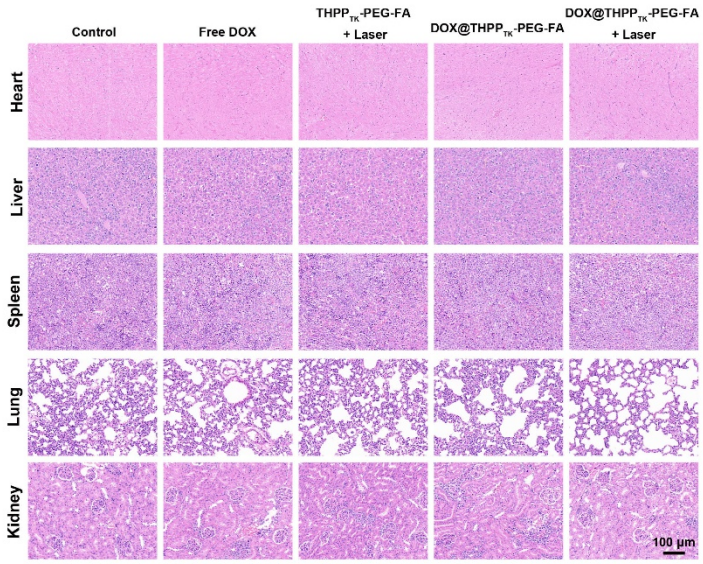

Figure S6. Histologic assessments of H&E staining in main organs of mice after treatment with different therapeutic formulations.