Electronic Supplementary Information for

Microporous Organic Network Nanoparticles for Dual Chemo-Photodynamic Cancer Therapy

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Experimental Sections

SEM images were obtained using a JSM6700F equipment. Normal TEM images and EDS elemental mapping images were obtained using a JEOL 2100F. The surface areas of the MON materials were measured through analyzing N_2 adsorption-desorption isotherm curves obtained at 77K using a Micromeritics ASAP2020 and a BELSORP II-mini equipment. The pore size distribution diagrams were obtained by the density functional theory method. IR absorption spectra were obtained using a Bruker VERTEX 70 FT-IR spectrometer. Solid state ^{13}C NMR spectra were obtained on CP/TOSS mode using a 500 MHz Bruker ADVANCE II NMR spectrometer at the NCIRF of Seoul National University. Reflectance spectroscopy was conducted using a SHIMADZU UN-3600. The absorption spectra of N-SMON and ZnPhT/N-SMON were obtained through conversions of their reflectance spectra. UV/vis absorption spectra were obtained using a JASCO V-630 and an Optizen 3220UV (Mecasys Co., Ltd, Daejeon, Korea) spectrometers. TGA curves were obtained under N_2 using a Seiko Exstar 7300. PXRD patterns were obtained using a Rigaku MAX-2200. Elemental analysis was conducted using a CE EA1110 analyzer. The water contact angles were measured using a Theta Optical Tensiometer model (KSV instruments, Ltd.) and an electrooptics setup comprising a CCTV camera connected to a computer (software Attension Theta). Zeta potentials were measured using a Zetasizer ZS90 (Malvern).

Synthesis of ZnPhT/N-SMON

For the preparation of N-MON nanoparticles, poly(vinylpyrrolidone) (PVP, Mw: 40000, 1.86 g) was dissolved in ethanol (60 mL) by sonication. Under argon, $(PPh_3)_2PdCl_2$ (25 mg, 36 μmol) and CuI (6.9 mg, 36 μmol) were added to a flame-dried 100 mL Schlenk flask. After triethylamine (30 mL) and the PVP solution described above were added, the reaction mixture was sonicated for 40 min. Tetra(4-ethynylphenyl)methane was prepared by the synthetic procedures reported in the literature.[1] After tetra(4-ethynylphenyl)methane (0.15 g, 0.36 mmol) and 1,4-diiodobenzene (238 mg, 0.72 mmol) were added, the reaction mixture was heated with stirring at 100°C for 24 h. After being cooled to room temperature, the precipitates were retrieved by centrifugation, washed with methanol and methylene chloride three times each, and dried under vacuum.

For the preparation of N-SMON nanoparticles, N-MON (0.20 g) and distilled methylene chloride (40 mL) were added to a 100 mL flame-dried Schlenk flask under argon. After the reaction mixture was cooled to 0°C with an ice bath, chlorosulfonic acid (2.6 mL, 39 mmol) was slowly added. *Caution: a glass stopper is more suitable than a conventional rubber septum, because the rubber septum can be chemically decomposed by possible acidic vapor.* The reaction mixture was stirred at room temperature for 1.5 h. Methanol (~20 mL) was then slowly added to the reaction mixture at 0°C to quench excess chlorosulfonic acid. The resultant solid was retrieved by centrifugation, washed a mixture of water (20 mL) and methanol (25 mL) until the washing solution became neutral, and dried under vacuum.

For the preparation of ZnPhT/N-SMON nanoparticles, ZnPhT (refer to the structure in shown Figure 1) was prepared by the synthetic procedures described in the literature.[2] In a 30 mL vial, after N-SMON (50 mg) and distilled water (30 mL) were added to a 30 mL vial, the reaction mixture was sonicated for 30 min. After ZnPhT (25 mg, 16 μmol) was added, the reaction mixture was sonicated for 30 min. The resultant solid was separated by filtration, washed with distilled water (100 mL), methanol (150 mL), and acetone (100 mL), and dried under vacuum.

Measurement of the singlet oxygen generation (SOG) efficiency of ZnPhT/N-SMON

The singlet generation ability of ZnPhT/N-SMON was studied using a 9,10-dimethylanthracene (DMA, Aldrich Co.) as a fluorescent probe. By diluting the stock solution of DMA (1.0 mg, 4.8 μmol) in DMF (1 mL), 3.9 μM DMA solution in DMF (1 mL) was prepared. By diluting the stock mixture of ZnPhT/N-SMON (10 mg) in DMF (1 mL), the suspensions of ZnPhT/N-SMON (40, 80, 120, and 160 μg/mL) in DMF (1 mL) were prepared. The DMA solution were mixed with the ZnPhT/N-SMON suspension. After a laser (λ = 671 nm, light intensity of 50 mW/cm^2, fiber-coupled laser system, LaserLab®, Korea) was irradiated for 80 s, the emission intensity of DMA was measured at 436 nm (λ_{ex} = 375 nm) by a spectrofluorophotometer (RF-5301; Shimadzu).

Experimental procedures for DOX loading on the ZnPhT/N-SMON

ZnPhT/N-SMON (10 mg) was suspended in a 1:1 mixture (10 mL) of DMSO and distilled water. Doxorubicin hydrochloride (10 mg, 0.017 mmol) was treated with triethylamine (3.6 μL, 0.026 mmol) in DMSO (1 mL) overnight.[3] After DOX (1.0 mg, 1.8 μmol) in DMSO was added to the suspension of ZnPhT/N-SMON, the mixture was stirred in a dark room overnight. The mixture was transferred to a dialysis tube (MWCO, 3.5 kDa), dialyzed against distilled water for 24 h, and lyophilized to form DOX/ZnPhT/N-SMON. To measure the amount of DOX loading, the DOX/ZnPhT/N-SMON was sonicated in a 8:2 mixture of DMSO and distilled water in a dark room. The emission intensity of DOX was measured at 590 nm (λ_{ex} = 490 nm) using a microplate reader (Bio-Tek, VT, USA) and the amount of DOX was calculated based on a calibration curve. The loading efficiency (LE) and the loading contents (LC) were calculated using the following equations. LE (%) = [DOX_{en}/DOX_{total}] × 100, LC (%) = (DOX_{en}/TM) × 100, DOX_{en}: the amount of DOX in DOX/ZnPhT/N-SMON, DOX_{total}: the feed amount of DOX for the preparation of DOX/ZnPhT/N-SMON, TM: the amount of DOX/ZnPhT/N-SMON.

In vitro DOX release profile of DOX/ZnPhT/N-SMON

DOX/ZnPhT/N-SMON (1 mg) was dispersed in a distilled water (1 mL) and transferred to a dialysis tube (MWCO, 3.5 kDa). The dialysis tube was added to phosphate buffered saline solution containing 0.1% Tween 20 (PBST, pH 7.4, 10 mL) and incubated in a shaking water bath (100 rpm) at 37°C in a dark room. At predetermined times (1, 2, 4, 8, 12, 24, 48, and 72 h), the outer solution was exchanged with a fresh PBST buffer (10 mL). The emission intensity of DOX in the solution was measured at 590 nm (λ_{ex} = 490 nm) by a microplate reader (Bio-Tek, VT, USA) and the released amounts of DOX were calculated from a calibration curve.

Cell culture and incubation conditions

HCT-116 (human colon cancer) cells were obtained from the Korean Cell Line Bank. The RPMI 1640 medium supplemented with 10% heat inactivated fetal bovine serum (FBS) and 1% penicillin/streptomycin (called a complement medium, denoted CM in this study) was prepared. RPMI-1640 medium, fetal bovine serum (FBS), and antibiotics (penicillin/streptomycin) were obtained from GibcoBRL (Invitrogen Corp., Carlsbad, CA, U.S.A.). The HCT-116 cells were cultured in the CM as monolayers at 37°C with 100% humidity and 5% CO_2 and sub-cultured in new media every 1-2 days. ZnPhT/N-SMON and DOX/ZnPhT/N-SMON were dispersed in serum-free (SF) medium. In the case of DOX only, the DOX(10 mg, 0.018 mmol) was dissolved in DMSO (1 mL) and diluted in SF medium until the content of DMSO became <0.1%. Untreated cells were kept in the dark room and used a reference standard.

In vitro cellular uptake test

To confirm the cellular uptake of DOX/ZnPhT/N-SMON, HCT-116 cells were seeded in a 6-well plate with a density of 1×10^5 cells per well and incubated at 37°C in 5% CO_2 for 24 h. The culture medium was replaced with the DOX/ZnPhT/N-SMON suspensions (20, 40, 60, and 80 μg/mL) in SF medium. After being incubated for 4 h, the cells were rinsed with Dulbecco's phosphate-buffered saline (DPBS, GibcoBRL, Invitrogen Co.) two times and resuspended in the DPBS. The degrees of cellular uptake were analyzed by flow cytometry with a Becton-Dickinson FACS Canto Ⅱ (San Jose, CA, USA). For each sample, 10,000 cells (gate events) were measured and the emissions of DOX/ZnPhT/N-SMON were measured by logarithmic settings (Alexa-Fluor 647, λ_{em} = 670 nm, PE, λ_{em} = 578 nm). The results were analyzed statistically using a FACS Diva software (BD). To characterize the cellular localization of DOX/ZnPhT/N-SMON, HCT-116 cells were seeded on the coverslip in a 6-well plate (a density of 1×10^5 cells per well) using CM (2 mL) and incubated for 24 h. The incubation medium was replaced with a suspension of DOX/ZnPhT/N-SMON in SF medium. After being incubated for 4 h, the cells were rinsed with DPBS two times, fixed with 4% paraformaldehyde, and stained with DAP. The cells were investigated by confocal laser scanning microscope (LSM 710 Meta, Carl Zeiss, Germany). The fluorescence images were analyzed using a LSM image browser software (Zen series, Carl Zeiss, Germany).

In vitro cytotoxicity assay

Considering the non-cytotoxic concentrations (without laser irradiation), the PDT performance of ZnPhT/N-SMON was studied at a concentration range of 20 ~ 80 μg/mL. The HCT-116 cells were seeded on 35 mm cell culture plates (the density of 3×10^5 cells per well) and incubated for 24 h. After the cells were treated with ZnPhT/N-SMON suspensions (20, 40, 60, and 80 μg/mL) in SF medium (1 mL) for 4 h, the incubation medium was removed. After being rinsed with DPBS two times, the cells were added to CM. Each well was irradiated with a laser (λ = 671 nm, light intensity of 50 mW/cm^2, fiber-coupled laser system, LaserLab®, Korea) for 80 s. After the cells were incubated for 24 h, the formed formazan crystals were dissolved in DMSO and transferred to a new plate. The absorbance was measured at 570 nm using a microplate reader (Bio-Tek, VT, USA).

To study the dual PDT and chemotherapy effect, HCT-116 cells were seeded in 35 mm cell culture plates at the density of 3×10^5 cells per well and incubated for 24 h. After the incubation medium was removed, the cells were treated with DOX (5.0 μg/mL), ZnPhT/N-SMON (56.7 μg/mL), and DOX/ZnPhT/N-SMON (61.7 μg/mL) in SF medium (1 mL) and incubated for 4 h. After the plates were rinsed with DPBS two times, CM (2 mL) was added to each well. After the laser (λ = 671 nm, light intensity of 50 mW/cm^2, irradiation energy of 4 J/cm^2, fiber-coupled laser system, LaserLab®, Korea) was irradiated, the cells were incubated for 48 h. The formed formazan crystals were dissolved in DMSO, the absorbance was measured at 570 nm using a microplate reader (Bio-Tek, VT, USA).

In vivo studies of antitumor efficacy and organ toxicity

To investigate the antitumoral chemo-PDT effect, 5-week BALB/c male mice were inoculated with 5×10^7 HCT-116 cells into the right flank and randomly grouped when the tumor volume reached to 50 mm^3 (n = 5). The PBST (100 μL), free DOX (2.5 mg/Kg), ZnPhT/N-SMON (28.4 mg/Kg), and DOX/ZnPhT/N-SMON (30.9 mg/Kg) were intratumorally injected twice to the mice. After 24 h, the tumor regions of ZnPhT/N-SMON and DOX/ZnPhT/N-SMON groups were irradiated by a 671 nm laser (light intensity of 250 mw/cm^2, fiber-coupled laser system, LaserLab®, Korea) for 400 s. Tumor volume and weights of mice were monitored 3-4 times per week. The tumor volume was measured using a digital caliper by a formulation $V = L \times W^2/2$ (V, tumor volume; L, tumor length; W, tumor width).

At the end of the animal studies, mice were sacrificed and major organs (liver, lung, spleen, heart and kidney) were retrieved and fixed in 4% paraformaldehyde. Organ tissues were embedded with OCT compound, sectioned into 10 μm thick slices, stained with hematoxylin and eosin (H&E), and imaged using a slide scanner (APERIO CS2, Leica, Germany).

References

1. S. Yuan, S. Kirklin, B. Dorney, D. -J. Liu and L. Yu, *Macromolecules* 2009, **42**, 1554-1559.

2. I. Scalise and E. N. Durantini, *Bioorg. Med. Chem.* 2005, **13**, 3037-3045.

3. H. Park, W. Park and K. Na, *Biomaterials* 2014, **35**, 7963-7969.

Fig. S1 (a) Zeta potentials and (b) hydrodynamic diameters (the intensity-based diameters) of N-MON, N-SMON, ZnPhT/N-SMON, and DOX/ZnPhT/N-SMON in water.

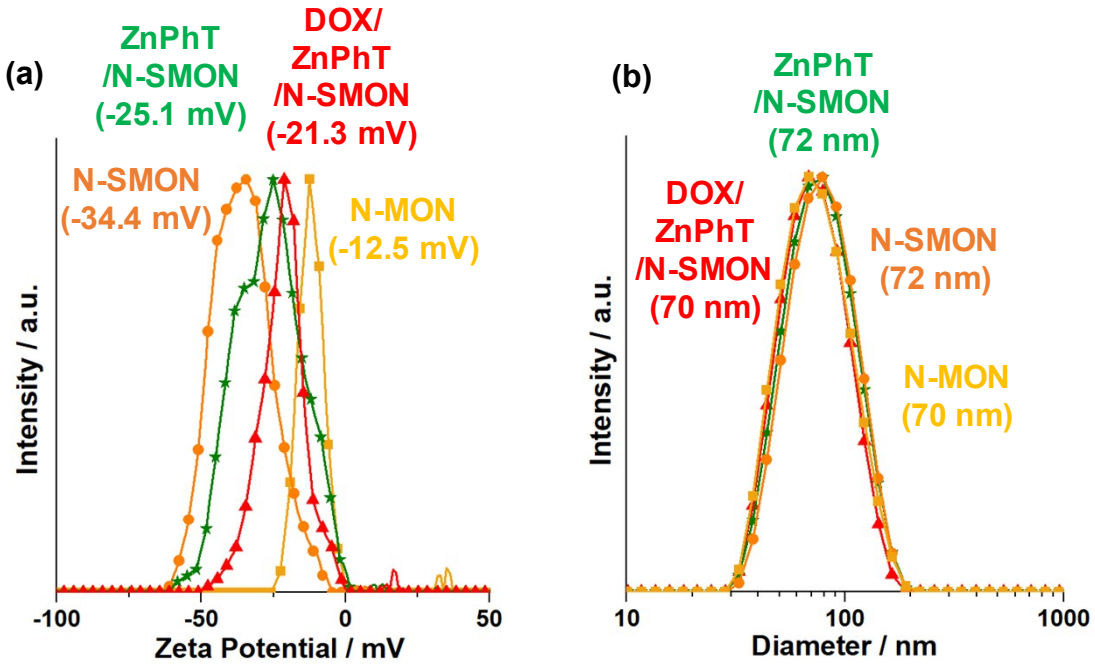

Fig. S2 PXRD patterns of N-MON, N-SMON, and ZnPhT/N-SMON materials.

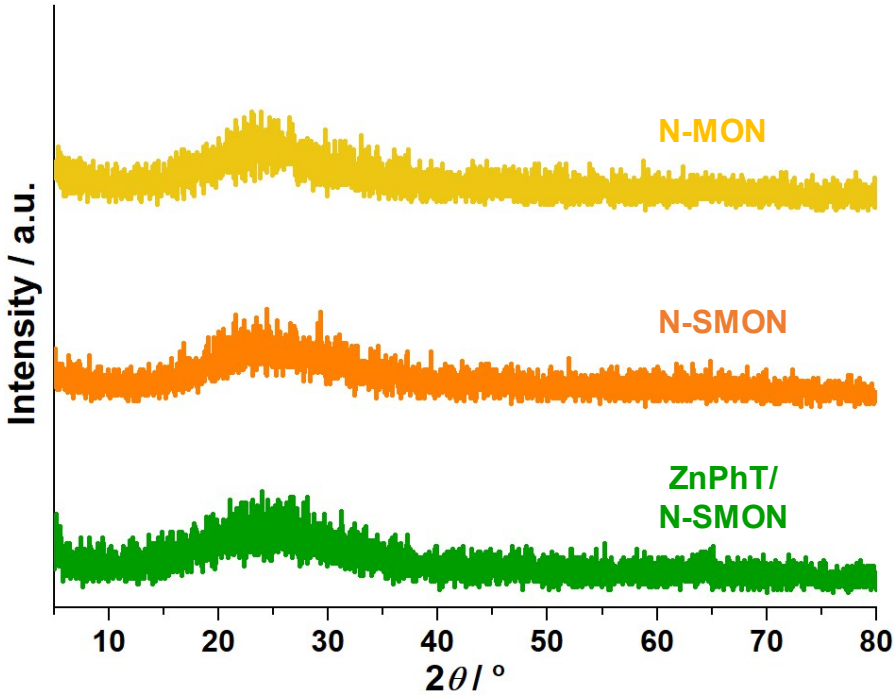

Fig. S3 The photographs of suspensions of N-MON, N-SMON, ZnPhT/N-SMON, and DOX/ZnPhT/N-SMON in water (0.50 mg/ 7 mL) before and after one week.

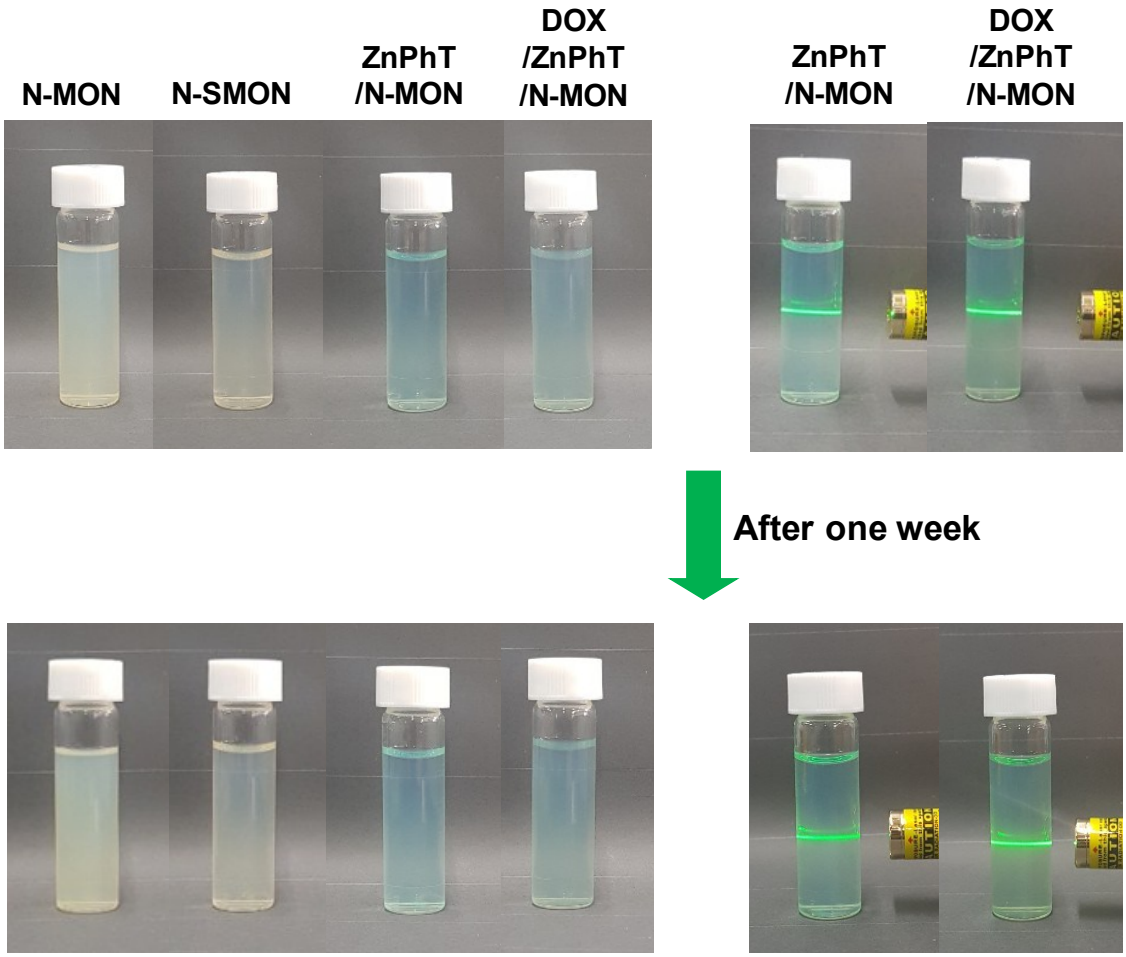

Fig. S4 The changes of UV/vis absorption spectra of PBST solution with the suspended ZnPhT/N-SMON (1.0 mg ZnPhT/N-SMON in 10 mL PBST, 37°C) for one week.

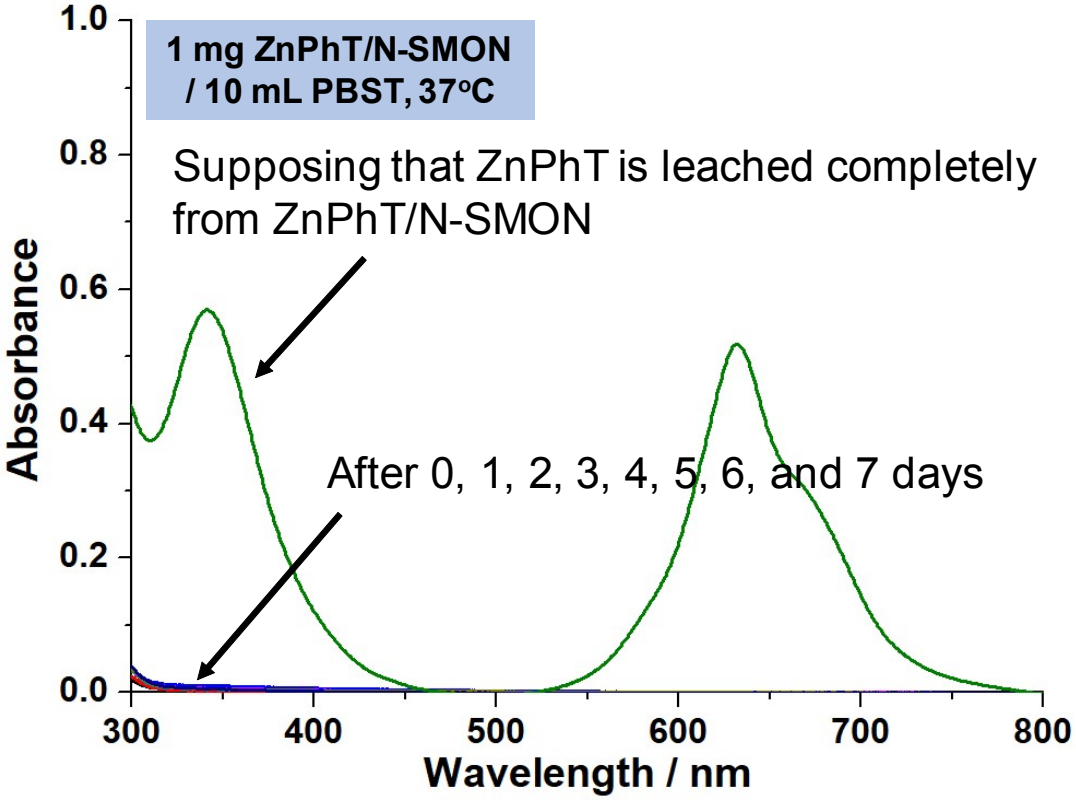

Fig. S5 (a) The changes of UV/vis absorption spectra of model blood solution (T. Kokubo *et al. J. Biomed. Mater. Res.* 1990, **24**, 721-734.) with the suspended ZnPhT/N-SMON (1.0 mg ZnPhT/N-SMON in 10 mL model blood solution, 37°C) for one week and (b) TEM images of ZnPhT/N-SMON before and after a week.

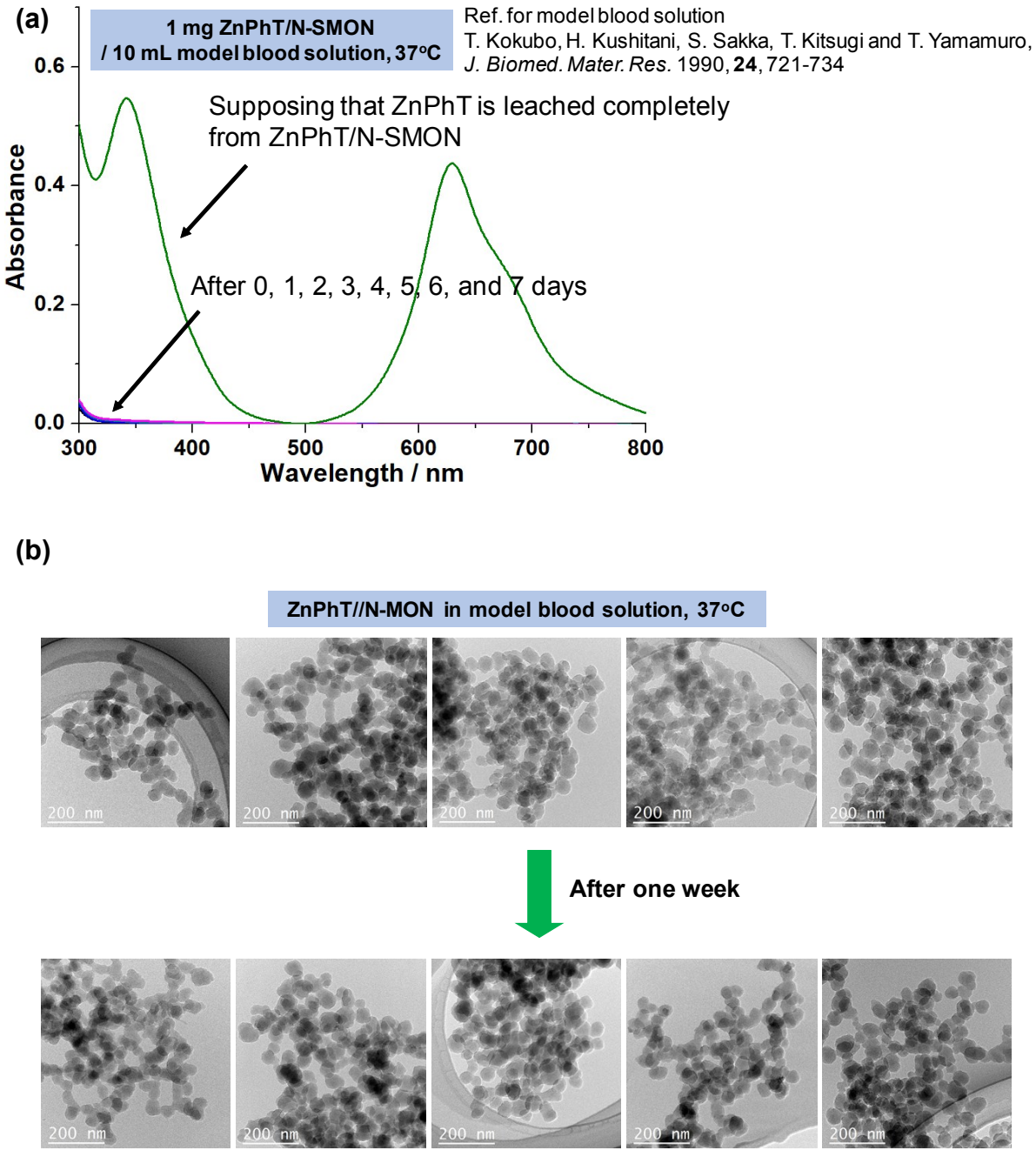

Fig. S6 (a) Laser (λ = 671 nm)-induced singlet oxygen generation performance of ZnPhT/N-SMONs based on the emission quenching of 9,10-dimethylanthracene (DMA) in PBST (pH 7.4) buffer solution. (b) Laser irradiation (λ = 671 nm) energy dependent PDT performance of ZnPhT/N-SMONs for cancer cells.

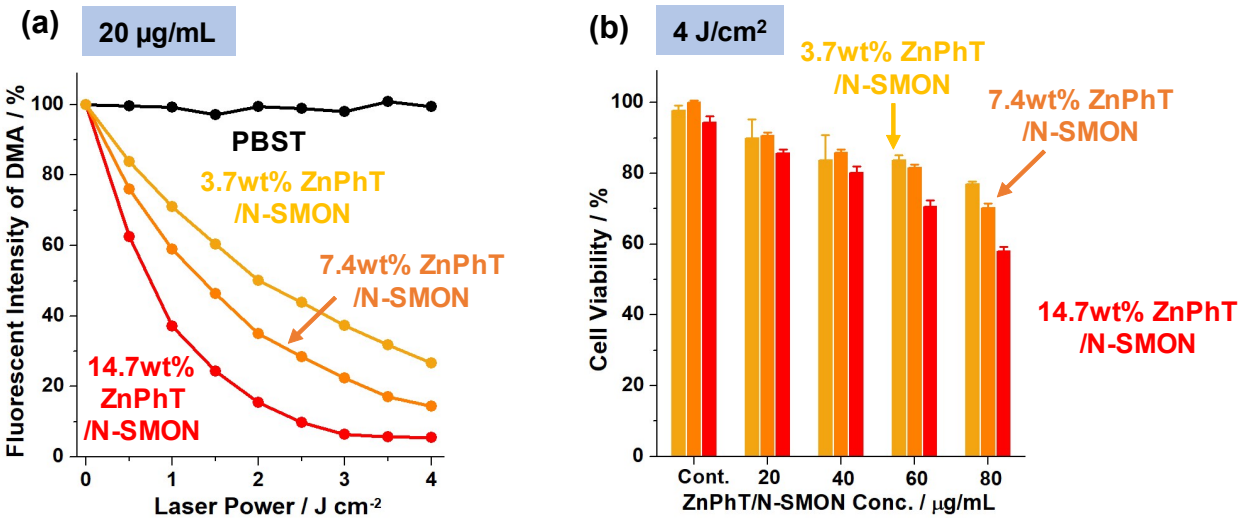

Fig. S7 The changes of body weights of mice after injection of PBST, free DOX (2.5 mg/Kg), ZnPhT/N-SMON (28.4 mg/Kg), and DOX/ZnPhT/N-SMON (30.9 mg/Kg) with/without a laser irradiation.

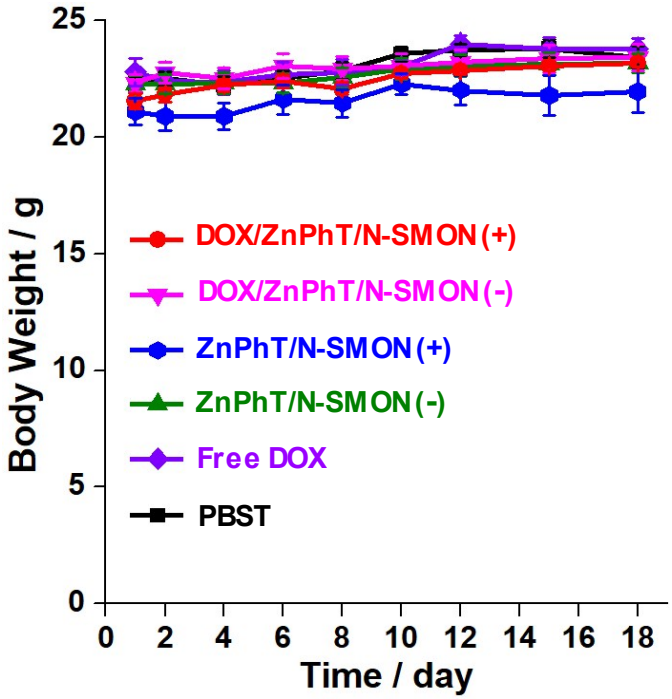

Fig. S8 Investigation of possible damages of major organs after in vivo tests (Balb/c mice, n=5) for the inhibition of tumor growth by ZnPhT/N-SMON (28.4 mg/Kg), and DOX/ZnPhT/N-SMON (30.9 mg/Kg) with/without a laser irradiation. Scale bars = 100 μm.