Supporting Information

pH-Responsive Dopamine-Based Nanoparticles Assembled via Schiff Base Bonds for Synergistic Anticancer Therapy

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Experimental Section

Materials

Dopamine hydrochloride (DA), 9,10-anthracenediyl-bis(methylene) dimalonic acid (ABDA), methanol, calcein acetoxymethyl ester (calcein-AM), tris(hydroxymethyl)aminomethane (Tris), and propidium iodide (PI) were purchased from Sigma Aldrich. Glutaraldehyde (GA) manganese sulfate ($MnSO_4$), ammonium hydrogen carbonate (NH_4HCO_3), ethanol, and ethylenediaminetetraacetic acid (EDTA) were obtained from Sinopharm Chemical Reagent Co., Ltd. Sodium hydroxide (NaOH) and dimethyl sulfoxide (DMSO) were purchased from Beijing Chemical Co., Ltd. Hoechst 33342, Alexa Fluor 488, and cell counting kit-8 (CCK-8) were supplied by Dojindo Laboratories, Kumamoto, Japan. Doxorubicin (DOX) was obtained from Shanghai Aladdin Bio-Chem Technology Co., Ltd. Chlorin e6 (Ce6) was purchased from J&K Chemical Ltd. (Shanghai, China). Phosphate buffered saline (PBS) tablets, Dulbecco's modified eagle medium (DMEM), and fetal bovine serum (FBS) were obtained from HyClone. Ultrapure water used in all experiments was prepared in a Milli-Q apparatus (Millipore), and had a resistivity higher than 18.2 MΩ·cm.

Characterization

The morphology and size of the nanoparticles were investigated by using scanning electron microscopy (SEM, Hitachi S-4800), transmission electron microscope (TEM, JEOL JEM-7700), and dynamic light scattering (DLS, Malvin Zetasizer Nano ZS). UV-Vis absorption spectra were recorded at ambient condition with the SHIMADZU-2600 spectrophotometer. Fourier transform infrared spectroscopy (FTIR) was conducted on a Tensor-27 infrared spectrometer (Bruker) with a KBr pellet pressing method. Confocal laser scanning microscopy (CLSM) images were acquired using the confocal microscope (Olympus FV1000). Mass spectrometry was analyzed by liquid chromatograph mass spectrometer (LC-MS, Thermo-Fisher Ultimate 3000-LCQ Fleet). A 660 nm laser was used as the light source, which was obtained from Changchun Leishi Photoelectric Co., Ltd.

Preparation of DG nanoparticles

The preparation of DG nanoparticles (DGNPs) was typically performed as follows: 0.5 mL 6 mM DA solution and 0.5 mL 0.12 wt% GA solution were mixed in a centrifuge tube under quick agitation. The pH of the mixture was adjusted to 6.7 by adding 0.1 M NaOH solution. Then, the mixture reacted at the room temperature under continuous magnetic stirring for 24 h. The light blue solution changed into yellow suspension, while the nanoparticles were prepared. the as-synthesized product was collected by 3 cycles of centrifugation (8000 rpm, 5 min) and water washing.

Preparation of polydopamine (PDA) microcapsule

$MnCO_3$ microparticles were synthesized with the co-precipitation method. Typically, $MnSO_4$ in water (0.016 M, 100 mL) was mixed thoroughly with 10 mL ethanol under ultrasound for 10 s. The resulting solution was mixed with NH_4HCO_3 in water (0.16 M, 100 mL) for 30 s. After that, the mixed solution was quiescent for 30 min. The $MnCO_3$ particles with an average size of 4.5 μm were obtained followed by triple rinsing with water.

For the preparation of PDA capsules, $MnCO_3$ microparticles acted as the sacrificial templates. $MnCO_3$ particles were resuspended in Tris-HCl buffer (10 mM, pH 8.5) of dopamine hydrochloride solution (1 mg mL^{-1}), followed by shaking for 12 h's reaction. Then, as-prepared particles with dark brown color were centrifuged (3000 rpm, 5 min) and washed for 3 times. Finally, the PDA capsules were acquired after the removal of $MnCO_3$ microparticles with EDTA solution (50 mM, pH 7.4).

Stability of DGNPs in different conditions

The stability of DGNPs in different solutions was investigated by SEM and TEM. 100 μL of DGNPs (1 mg/mL) suspension was respectively added into phosphate buffer saline (pH 7.2) and HCl solutions with various pH values (6.0, 5.0, and 4.0) and then incubated for 7 days.

Preparation of DGNPs@DOX/Ce6 complex

0.5 mL 6 mM DA solution and 0.5 mL 0.12 wt% GA solution were mixed in a centrifuge tube under quick agitation. The pH of the mixture was adjusted to 6.7 by adding 0.1 M NaOH solution, and the mixture reacted at the room temperature under continuous magnetic stirring. After 2 h's aging, the solution turned yellow. Then, 50 μL of DOX solution (1 mg/mL) and 50 μL of Ce6 solution (1 mg/mL) were added into the solution, and the mixture was stirred for 22 h to synthesize DGNPs@DOX/Ce6 complex. As-obtained DGNPs@DOX/Ce6 complex was collected by 3 cycles of centrifugation (8000 rpm, 5 min) and water washing.

Drug release of DGNPs@DOX complex

To assess the drug release behavior, the DGNPs@DOX complex was dispersed in 1.8 mL of different buffer solutions (pH 7.2 and pH 5.2). The amount of DOX released from the nanoparticles was monitored on the basis of the UV-Vis absorbance of the supernatant at 485 nm, and the cumulative amount of released drug was calculated. The percentage of drug released from DGNPs@DOX complex was plotted against time, with the maximum amount of the drug released in the given time set as 100%.

Detection of $^1O_2$

For the detection of $^1O_2$ generation, 9,10-Anthracenediyl-bis(methylene) dimalonic acid was employed as the chemical probe. ABDA stock solution (10 μM) was added to the DGNPs@DOX/Ce6 complex aqueous solution. Then, the mixed solution was irradiated with 660 nm laser (500 mW/cm^2) for varied time. The decrease of the adsorption at 378 nm upon the reaction of ABDA with $^1O_2$ was measured with UV-Vis spectrophotometry.

***In vitro* cellular uptake**

HeLa cells were cultured in DMEM supplemented with 10% FBS, 1% penicillin, and 1% streptomycin at 37 °C in a humidified environment of 5% CO_2 for 24 h. The medium was replaced with 1 mL DMEM medium with as-prepared DG nanoparticles, and then incubated for 7 h. After that, HeLa cells were washed with PBS for 3 times, and 1 mL DMEM medium was added. 10 μL Alexa 488 (1 mg/mL) and 10 μL Hoechst 33342 (1 mg/mL) were taken to

stain the cell membrane and nuclei, respectively. The intracellular localization of the nanoparticles was observed with CLSM equipped with a UPLSAPO 60× objective.

Cytotoxicity assay

HeLa cells were seeded into the 96-well plate with a density of 1×10^4 cells per well and incubated in DMEM at 37 °C for 24 h. Then, cell culture media was removed and replaced by 100 μL DMEM in the presence of serum containing equivalent concentrations of DGNPs, free DOX, free Ce6, and DGNPs@DOX/Ce6 complex, respectively. After the co-incubation with drugs for 7 h, the cells were washed with PBS for 3 times, and 100 μL DMEM medium was added. Some cells were irradiated with a 660 nm laser (500 mW/cm^2) for 5 min. After another 20 h's incubation, the cell viability was measured by using a CCK-8 assay.

Cytotoxicity was further studied with CLSM. HeLa cells were seeded into confocal dishes and treated with DGNPs and DGNPs@DOX/Ce6 complex for 7 h in the incubator, respectively. After that, the cells were washed with PBS for 3 times. 10 μL PI (1 mg/mL) and 10 μL calcein-AM (20 μg/mL) were added into the dishes. Then, the cells were treated with 660 nm laser (500 mW/cm^2) for 5 min and continued to be cultured for another 12 h. Cells cultured without drugs and/or irradiation were taken as the control groups.

***In vivo* biodistribution**

All animal experiments were performed in compliance with the relevant laws and institutional guidelines, and approved by the local ethics committee. Female BALB/c nude mice (18~20 g) were monitored *in vivo* by fluorescence imaging system. To establish the model of tumor, 100 μL of 1×10^7 MCF-7 cells were administered by subcutaneous injection into the right leg area of mice. When the diameter of tumor reached approximately 4-6 mm, 5% glucose solution of assembled DGNPs@DOX/Ce6 (equivalent Ce6 4.0 mg kg^{-1} body) was injected into the tumor bearing mice via the tail vein. At different time intervals, the mice were anesthetized with isoflurane and observed using an *In Vivo* Imaging System (Kodak In-Vivo imaging system FX Pro). The 650 nm pulsed laser was excited and the fluorescence was collected within 660-

690 nm. At 48 h after the injection, the mice were sacrificed and the organs (heart, liver, spleen, lung, and kidneys) and tumors were harvested for imaging.

***In vivo* tumor inhibition**

MCF-7 tumor-bearing BALB/c mice models were made by the same methods as above for the imaging studies. BALB/c mice (six to seven weeks) were randomized into 3 groups (4 mice in each group): control, DGNP@DOX/Ce6, and DGNPs@DOX/Ce6+Light. When the diameter of tumor reached 4-6 mm, the mice were injected with 5% glucose solution and 5% glucose solution of assembled DGNPs@DOX/Ce6 (100 μL 4 mg/mL) individually in situ (n = 4). For the DGNPs@DOX/Ce6+Light group, the tumor site of mice was irradiated with 660 nm laser (300 mW/cm^2) for 10 min. Then, the therapeutic efficacy of treated mice was evaluated by tumor volumes, which was calculated as follows: $V = 0.5 \times length \times width^2$. The tumor volume and the body weight were recorded everyday for 9 days.

Supplementary Figures

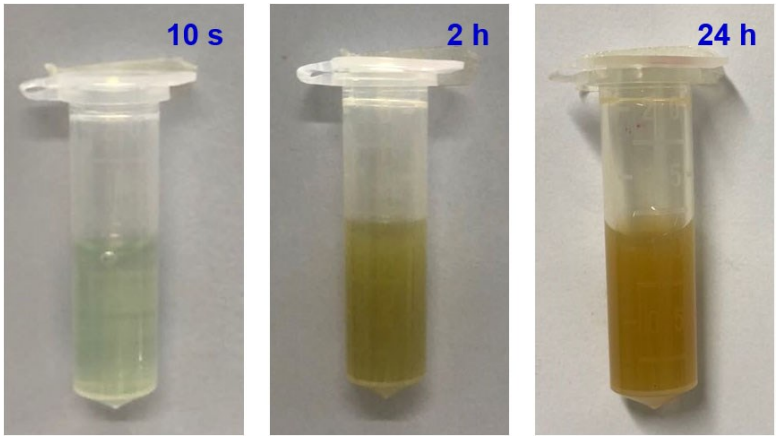

Figure S1 The photographs of DA and GA mixture at different reaction time.

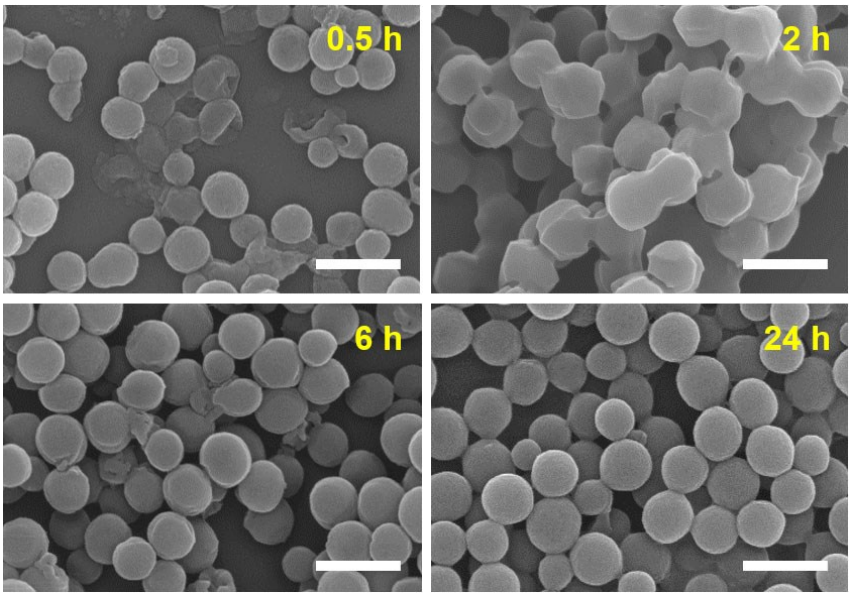

Figure S2 SEM images of DGNPs obtained at different reaction time. The scale bars represent 500 nm.

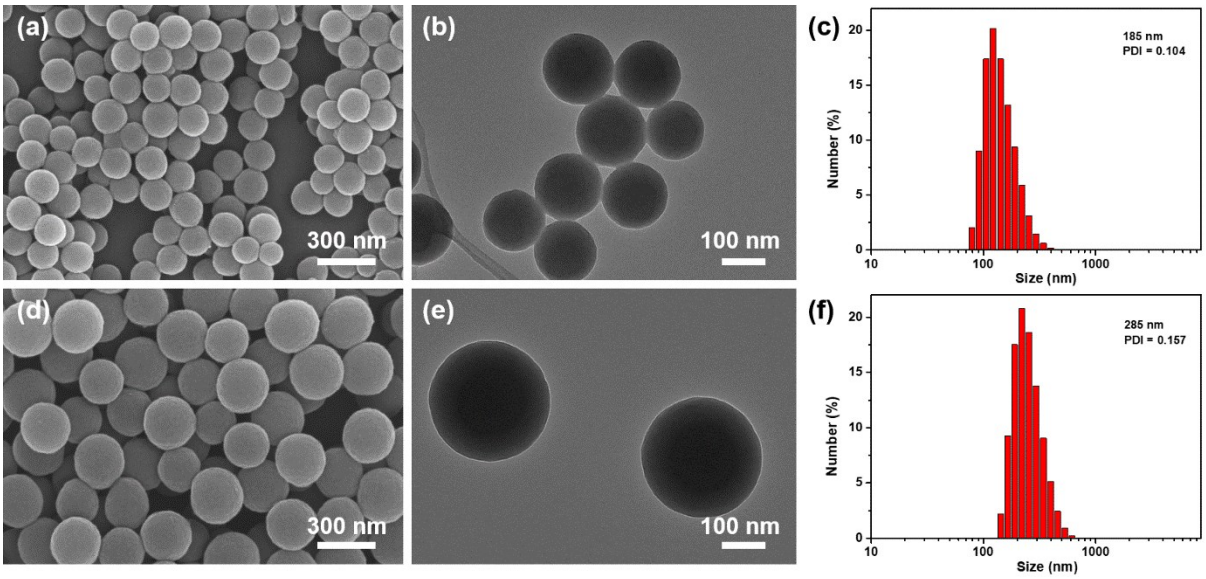

Figure S3 SEM images, TEM images and dynamic light scattering measurement of DGNPs obtained at different concentration of DA and GA. (a-c) 3 mM DA solution and 0.06 wt% GA solution, (d-f) 6 mM DA solution and 0.12 wt% GA solution.

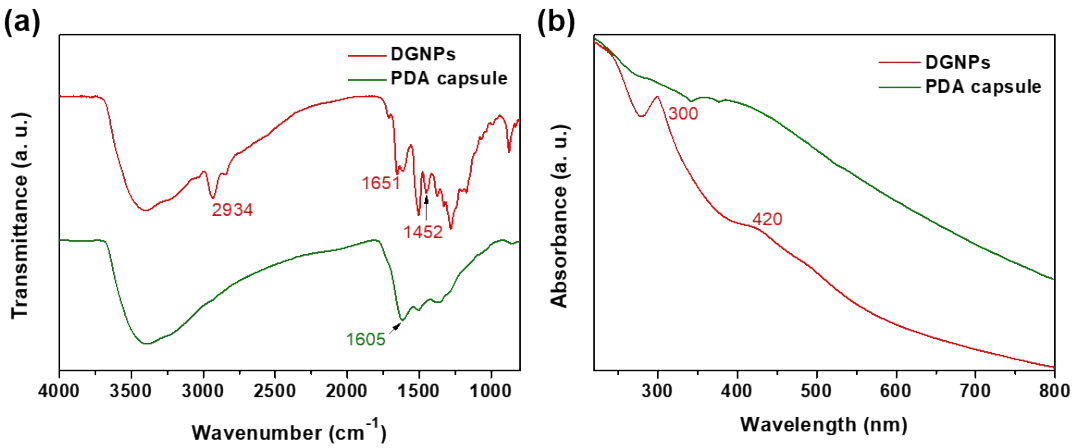

Figure S4 (a) FTIR spectra and (b) UV-Vis spectra of DGNPs and PDA capsules.

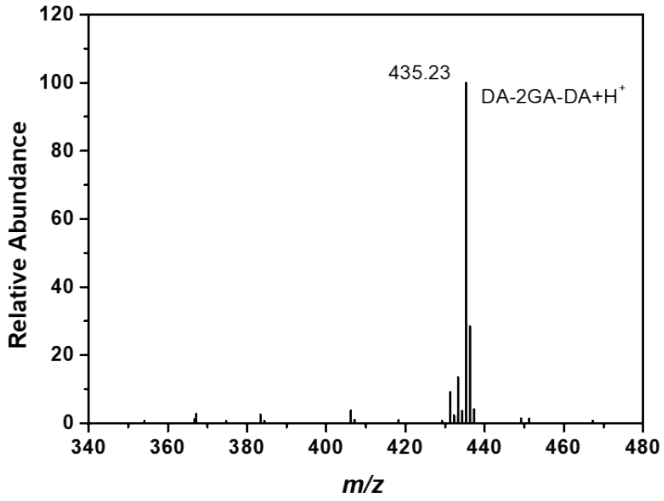

Figure S5 Mass spectrometry of DGNPs dissolved in methanol.

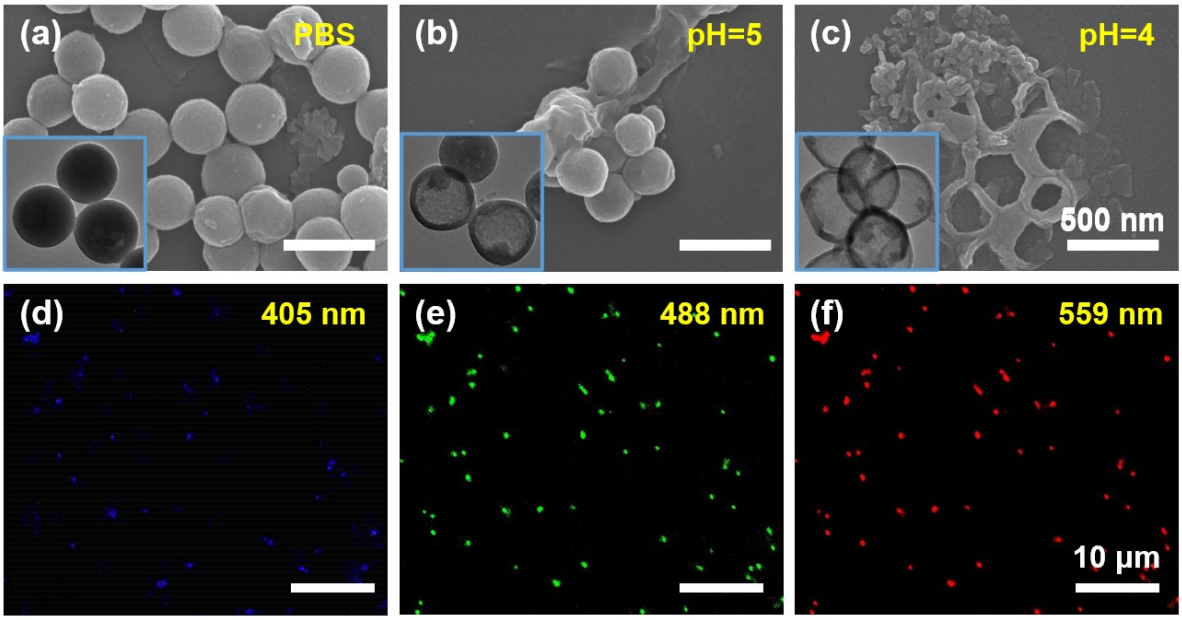

Figure S6 (a-c) SEM images and inserted TEM images of assembled DGNPs kept in PBS and different pH solutions for 7 days. (d-f) CLSM images of DGNPs.

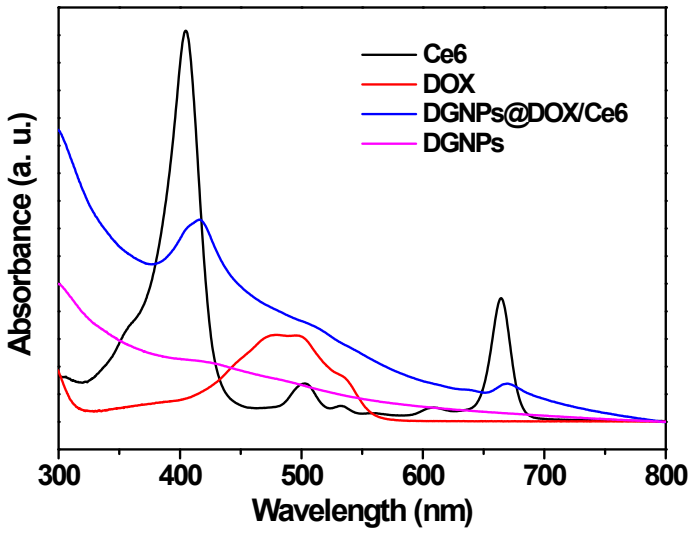

Figure S7 UV-Vis absorption spectra of Ce6, DOX, DGNPs, and DGNPs@DOX/Ce6 complex.

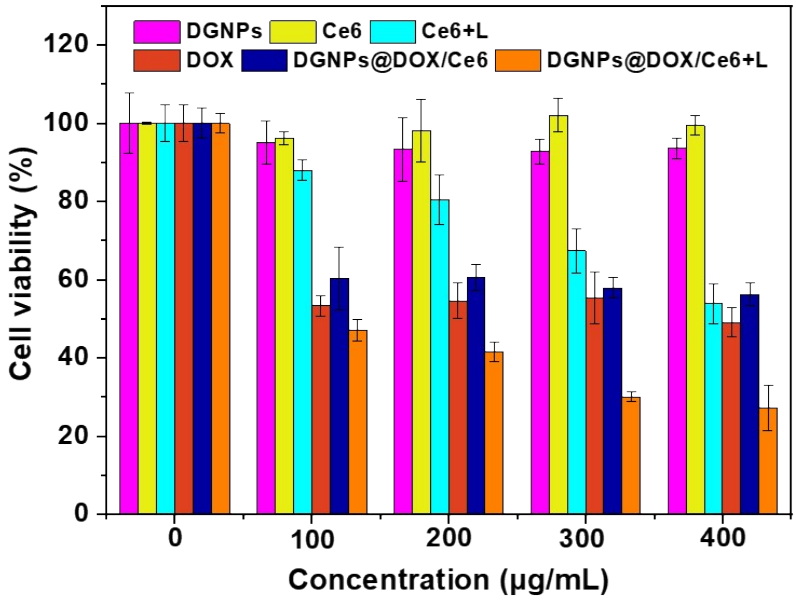

Figure S8 Cell viability of HeLa cells incubated under various treatments.

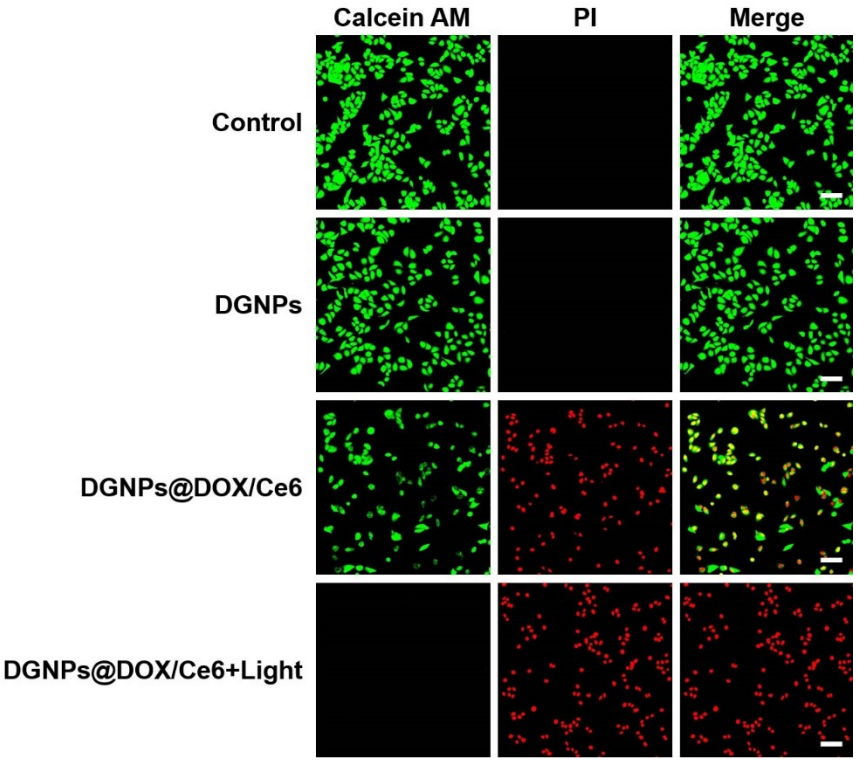

Figure S9 CLSM images of untreated HeLa cells (control group), HeLa cells treated with DGNPs, DGNPs@DOX/Ce6 without and with light irradiation (660 nm, 5 min). The samples were excited by 488 and 559 nm lasers. The scale bars represent 100 μm.

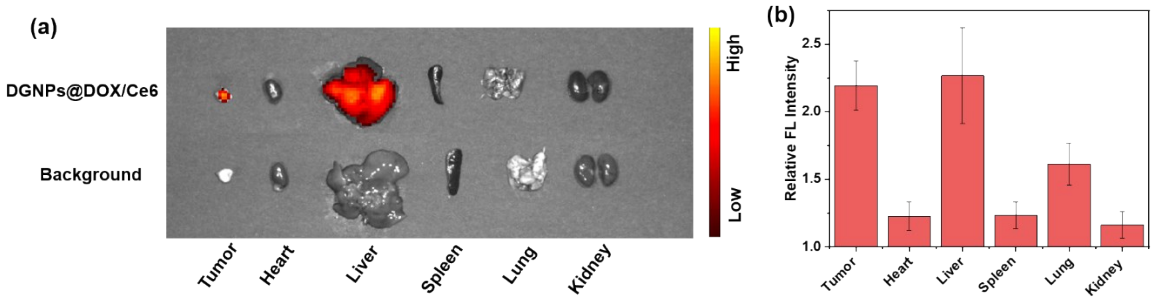

Figure S10 (a) *Ex vivo* fluorescence images of organs and tumors after 48 h post-injection of DGNPs@DOX/Ce6. (b) Quantification of fluorescence intensities in (a).

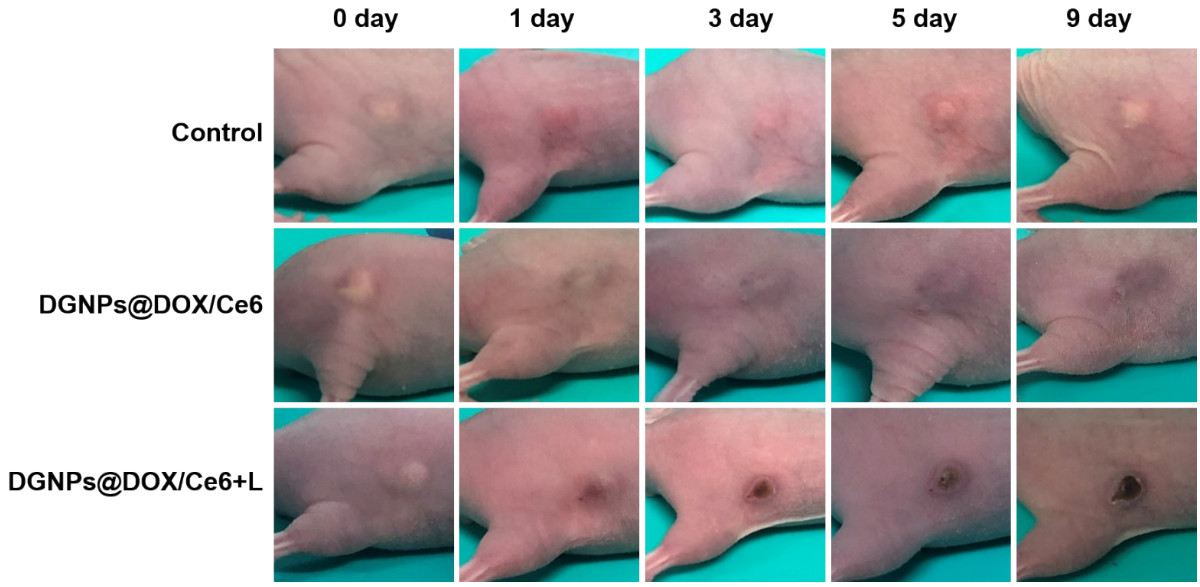

Figure S11 Representative photos of mice after various treatments at different time point.