Supporting information

1 Materials and methods

1.1 Materials

Ammonia bicarbonate (NH_4HCO_3) and calcium chloride dehydrate ($CaCl_2 \cdot 2H_2O$) were obtained from Sinopharm Chemical Reagent Co., Ltd. Dopamine hydrochloride was purchased from Sigma-Aldrich. Doxorubicin (DOX) was bought from Beijing Hua Feng United Technology Co. Ltd. 1, 2-dioleoyl-sn-glycero-3-phosphate (sodium salt) (DOPA) was obtained from Avanti Lipids Polar, Inc. 1, 2-dihexadecanoyl-sn-glycero-3- phosphocholine (DPPC), 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-(methoxy(polyethylene glycol)-5000) (DSPE-PEG_{5k}) and cholesterol were purchased from Xi'an Ruixi Biological Technology CO., Ltd. Fetal bovine serum (FBS) was purchased from Invitrogen. RPMI 1640 medium was purchased from Nanjing Key Gen Biotech. Co., Ltd., China.

1.2 Synthesis and characterization of DiR-DOX@$pCaCO_3$-PEG

$pCaCO_3$ nanoparticles were prepared by according to our previously developed method.[31] To prepare DiR-DOX@$pCaCO_3$-PEG nanoparticles, the obtained $pCaCO_3$ were mixed with DOX at a mass feeding ratio of 2:1 and kept at stirring for 4 h at room temperature. After removal of excess DOX via washing with ethanol the obtained DOX@$pCaCO_3$ were modified with a two-step assembly process as our previously reported.[30] Briefly, the DOX@$pCaCO_3$ ethanol solution (20 mg, 5 mL) was mixed with DOPA chloroform solution (2 mg, 1 mL) under the sonication treatment for 20 min. The obtained DOPA-coated DOX@$pCaCO_3$ nanoparticles after being purified by centrifugation at 14800 rpm were then re-suspended in 2 mL of chloroform containing cholesterol

(2 mg), DPPC (4 mg), and DSPE-PEG_{5k} (8 mg) and DiR (1.62 mg) under stirring overnight at room temperature. After being removed from the chloroform by using a rotary evaporator, the obtained dried lipid films were hydrated with PBS (2 mL) under sonication to prepare DiR-DOX@$pCaCO_3$-PEG. The purified liposomal nanoparticles were then stored at 4 ^oC for further use.

To obtain DiR-DOX-Gd@$pCaCO_3$-PEG, gadolinium chloride hexahydrate ($GdCl_3 \cdot 6H_2O$) ethanol solution (2 mg mL^{-1}, 1 mL) was mixed with $pCaCO_3$ (8 mg) dispersed in 2 mL of anhydrous ethanol, followed by being stirred for 1 h at room temperature. After removal of excess Gd^{3+} via washing with ethanol, the obtained Gd^{3+} doped $pCaCO_3$ (Gd^{3+}/$pCaCO_3$) were re-suspended in ethanol for future DOX loading and surface liposomal coating by according to the aforementioned procedure to prepare DiR-DOX-Gd@$pCaCO_3$-PEG. A FEI Tecnai F20 transmission electron microscope at an acceleration voltage of 200 kV (FEI company) was utilized to observe the morphology of the as-prepared nanoparticles. The UV-Vis-NIR spectra of these as-prepared nanoparticles and their DOX release profiles were acquired by using a GENESYS 10S (Thermo Scientific) UV-Vis-NIR spectrophotometer. A Malvern zetasizer (ZEN3690 zetasizer) was exploited to measure the hydrated diameters of diverse as-prepared nanoparticles. The contents of Gd^{3+} was determined by using an ICP-MS (aurora M90, Bruker, Germany).

1.3 Photothermal conversion capacity of DiR-DOX@$pCaCO_3$-PEG

To evaluate the photothermal conversion capacity of DiR-DOX@$pCaCO_3$-PEG, the temperature changing curves of DiR-DOX@$pCaCO_3$-PEG solution (50 μg mL^{-1}, in terms of DiR) under the irradiation of a 785-nm laser at 0.5, 0.75, and 1 W cm^{-2} were recorded by using an IR thermal camera (Fotric 225).

1.4 pH-responsive DOX release behaviors of DiR-DOX@pCaCO$_3$-PEG

To test the pH-responsive DOX release behaviors of DiR-DOX@pCaCO$_3$-PEG, the obtained DiR-DOX@pCaCO$_3$-PEG (DOX = 3 mg) were filled in the dialysis tubing with a molecular weight cut-off (MWCO) of 8000 Da and immersed in 15 mL phosphate buffer saline (PBS) at pH 5.5, 6.5, and 7.4. At designed time intervals, the released DOX concentrations in these dialysates were determined by recording its characteristic absorbance at 490 nm with a molar extinction coefficient of 10500 M^{-1} cm^{-1}.[33]

1.5 Cellular uptake of DOX@pCaCO$_3$-PEG

Murine triple negative breast 4T1 cells were obtained from the Cell Bank of Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, and then cultured with RPMI 1640 medium supplemented with 10% fetal bovine serum and 1% penicillin and streptomycin under a standard humidified condition (37 ^oC, 5% CO_2).

In order to explore the pH-dependent cellular behaviors of DOX@pCaCO$_3$-PEG, pre-seeded 4T1 cells in the 12-well plate (1*10^5 cells per well) were incubated with free DOX and DOX@pCaCO$_3$-PEG (5 μg mL^{-1}, in terms of DOX) at pH 6.5 and 7.4 containing for 4 h before bring washed twice with phosphate buffer solutions (PBS). Then, these treated cells were fixed with 4% paraformalclehyde solution for 20 min, and then stained with DAPI (1 μg mL^{-1}) for 10 min before mountained and observed using a confocal microscopy (Zeiss 800, Germany) by recording the intrinsic fluorescence of DOX molecules.

To explore the effect of laser irradiation on the cellular uptake of DiR-DOX@pCaCO$_3$-PEG, 4T1 cells pre-seeded in the 12-well plate (1*10^5 cells per well) were incubated with DiR-

DOX@pCaCO$_3$-PEG (DiR: 30 μg mL^{-1}) for 20 min in the presence or absence of 785 nm laser irradiation (0.5 W cm^{-2}). After being removed from the drug containing medium, these treated cells were incubated with fresh medium containing Lyso-tracker green (100 nM) for another 1 h at 37 ^{o}C before being fixed and subjected to confocal microscopic observation.

1.6 In vitro cytotoxicity study of DOX@pCaCO$_3$-PEG

To compare the cytotoxicity of free DOX and DOX@pCaCO$_3$-PEG towards 4T1 cells at both pH 6.5 and 7.4, 4T1 cells pre-seeded in the 96-well plate (1*10^4 cells per well) at pH 6.5 or 7.4 were incubated with both free DOX and DOX@pCaCO$_3$-PEG, respectively. After a 24 h incubation, these treated 4T1 cells subjected to the standard MTT assay for determining their remaining cell viability.

1.7 Photothermal effect enhanced cytotoxicity of DiR-DOX@pCaCO$_3$-PEG

To evaluate the effect of laser irradiation on promoting the cell killing capacity of DiR-DOX@pCaCO$_3$-PEG, 4T1 cells pre-seeded in the 96-well plate (1*10^4 cells per well) were incubated with free DOX, DiR@pCaCO$_3$-PEG and DiR-DOX@pCaCO$_3$-PEG (DOX: 5 μg mL^{-1}; DiR: 30 μg mL^{-1}) in the presence or absence of 785 nm laser irradiation for 20 min. After that, these treated 4T1 cells were cultured with fresh medium at 37 ^{o}C for another 24 h before their cell viabilities determined by using a standard MTT assay.

1.8 Animals experiments

Female Balb/c mice used in this study were obtained from Nanjing Peng Sheng Biological Technology Co., Ltd and used under the protocol approved by Soochow University Laboratory

Animal Center. To inoculate tumor models, subcutaneous 4T1 tumor models were established by subcutaneously injection corresponding cancer cells (2×10^6 cells) into the right back of each mouse.

To explore the tumor accumulation capacity of DiR-DOX@$pCaCO_3$-PEG, three mice bearing subcutaneous 4T1 tumors (~120 mm^3) were intravenously administrated with DiR-DOX@$pCaCO_3$-PEG (DiR: 2.5 mg kg^{-1}) before being anesthetized and imaged under an IVIS®Lumina III in vivo fluorescence imaging system (PerkinElmer) to record the DiR fluorescence intensity of tumor regions. At 48 h post injection (p.i.), these mice were sacrificed with their tumors and main organs including liver, spleen, kidney, heart and lung collected for ex vivo fluorescence imaging. Additionally, these organs and tumors were wet-weighted, homogenized and lysed before their DiR fluorescence determined using a microplate reader (Bio-tek, Synergy H1, USA).

To study the blood circulation behaviors of DiR-DOX@$pCaCO_3$-PEG, another 3 mice bearing subcutaneous 4T1 tumors were also intravenously injected with DiR-DOX@$pCaCO_3$-PEG and their blood sample (~25 μL) at varying time intervals were also collected and lysed with their DiR fluorescence determined using a microplate reader.

To study the capacity of DiR-DOX-Gd@$pCaCO_3$-PEG as in vivo MR imaging contrasting agent, another 3 mice bearing subcutaneous 4T1 tumors were also intravenously injected with DiR-DOX-Gd@$pCaCO_3$-PEG before being imaged under a SIEMENS skyra 3.0T MRI Scanner equipped with a special coil designed for small animal MR imaging. Besides, to evaluate the pH-responsive MR contrasting capacity of DiR-DOX-Gd@$pCaCO_3$-PEG, tumor bearing mice with simultaneous intratumoral and intramuscular injection of DiR-DOX-Gd@$pCaCO_3$-PEG were imaged under the aforementioned facility at 1 h post injection.

To evaluate the in vivo photothermal effect of DiR-DOX@$pCaCO_3$-PEG, a total of nine mice bearing subcutaneous 4T1 tumors (110 mm^3) was randomly divided into three groups (n = 3) and then intravenously administrated with PBS, DiR@$pCaCO_3$-PEG, and DiR-DOX@$pCaCO_3$-PEG, respectively. After 24 h p.i., these treated mice were exposed to a 785 nm laser irradiation for 20 min at 0.5 W/cm^2 with the temperature of the tumor region of each mouse recorded by using an IR thermal camera.

To evaluate the potency of laser irradiation on improving the chemotherapeutic efficacy of DiR-DOX@$pCaCO_3$-PEG, a total of 24 4T1 tumor bearing mice (110 mm^3) was randomly divided into four groups (n = 6) and treated as below: (1) PBS; (2) DiR@$pCaCO_3$-PEG + laser; (3) DiR-DOX@$pCaCO_3$-PEG; (4) DiR-DOX@$pCaCO_3$-PEG + laser. The intravenous injection doses of DOX and DiR were 5 mg kg^{-1} and 2.5 mg kg^{-1}, respectively. At 24 h p.i., mice with DiR@$pCaCO_3$-PEG or DiR-DOX@$pCaCO_3$-PEG injection were irradiated using a 785 nm laser with the tumor temperature kept at ~45 ^oC for 20 min. At one day after laser irradiation, one mouse of each group was sacrificed with the tumors harvested for H&E and TUNEL staining. Since the beginning of treatments, the length and width of each tumor were monitored every two days by using a Vernier caliper. The tumor volumes were calculated by following the formula of tumor volume = (width^2×length)/2. The body weight of each mouse during the treatment process was measured using an electronic balance. 12 days later, these mice were sacrificed and their tumors were collected, imaged using a digital camera, and weighted.

Table S1. The capacity of Ca^{2+}, DOX, and Gd^{3+} in the DOX-Gd@$pCaCO_3$ nanoparticles determined via different methods.

Components	Contents (%)	Quantification methods

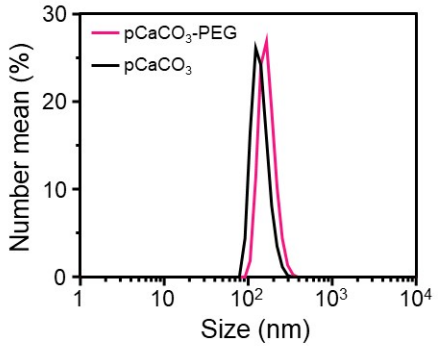
Ca	4.75 ± 0.14	Ca^{2+} detection kit
DOX	18.0 ± 0.42	UV-vis-NIR
Gd	2.49 ± 0.32	ICP-MS

Table S2. The raw data of MR intensity corresponding to Figure 4G analyzed via RadiAnt DICOM Viewer.

MR intensity (a.u.)						
	Muscle			Tumor		
0 min	156.08	169.03	165.86	195.29	197.27	194.6
60 min	145.29	143.19	144.01	258.68	276.88	281.05

Table S3. The raw data of MR intensity corresponding to Figure 4I analyzed via RadiAnt DICOM Viewer.

MR intensity (a.u.)			
Pre-injection	195.5	190.37	196.85
24 h	252.01	245.14	239.04



	Size (nm)	PDI
$pCaCO_3$-PEG	146.9	0.027
$pCaCO_3$	140.6	0.013

Figure S1. Hydrodynamic size distribution of $pCaCO_3$ and PEGylation $pCaCO_3$ nanoparticles.

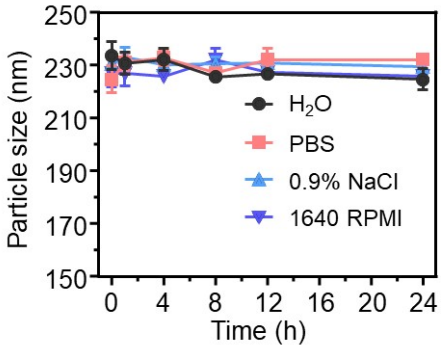


Figure S2. Time lapsed mean diameters of PEGylated DiR-DOX@$pCaCO_3$ incubated with different solutions for up to 24 h determined by DLS (n = 3). Data are presented as mean ± SD.

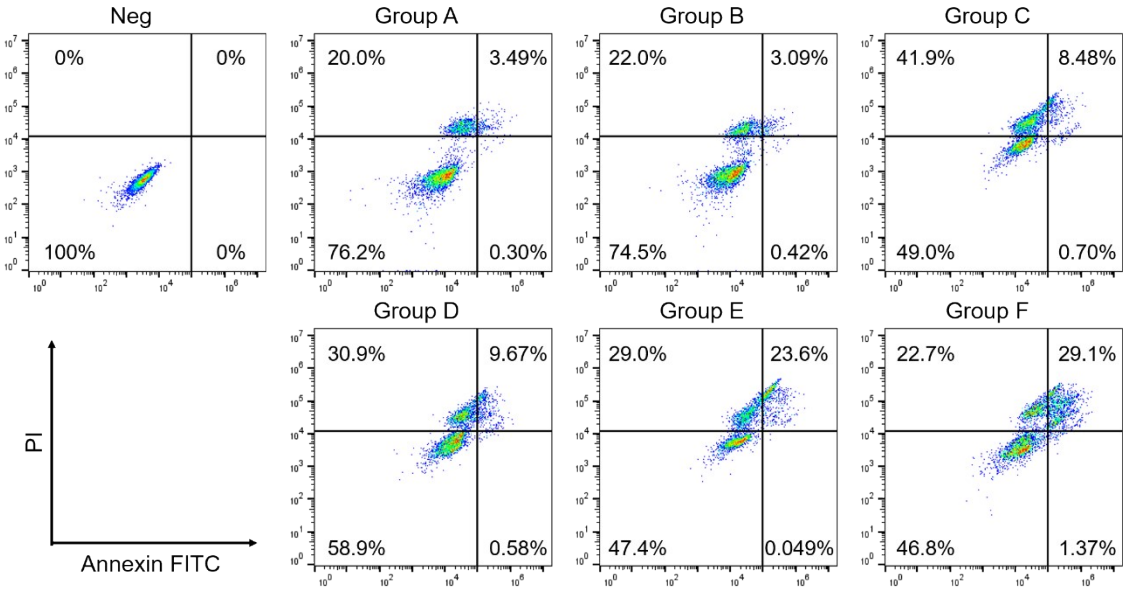


Figure S3. Flow cytometric analysis of apoptotic levels of 4T1 cells after incubation with Group A (DiR@$pCaCO_3$-PEG), Group B (DiR@$pCaCO_3$-PEG + irradiation exposure), Group C (free DOX), Group D (free DOX + irradiation exposure), Group E (DiR-DOX@$pCaCO_3$-PEG), and Group F (DiR-DOX@$pCaCO_3$-PEG + irradiation exposure) for 20 min and additional incubation without drugs for another 24 h (n = 3).

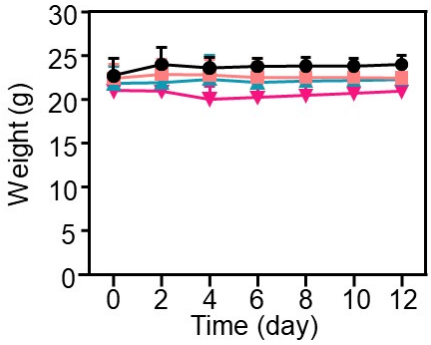


Figure S4. Body weight curves of four groups of mice recorded during the treatment process (n = 5).