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Electronic Supplementary Information

Self-assembly synthesis of polydiacetylene liposomes for thermo-triggered drug release and fluorescence tracking in cancer cells

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1: Preparation methods of PDA liposome and DTX-loaded PDA liposome.

Preparation of PDA liposome and DTX-loaded PDA liposome

PDA liposome and DTX-loaded PDA liposome (DLPLs) were prepared by self-assembly using a combination method of thin-film hydration and supercritical CO_2 fluid ($scCO_2$).[1] PCDA and egg lecithin (4:10, w/w) or PCDA, DTX, and egg lecithin (4:1:10, w/w/w) were weighed and dissolved in diethyl ether-ethanol (3:1, v/v) within a round-bottomed flask. Then the solvent was evaporated under reduced pressure using a rotary evaporator at 25°C till a uniform translucent film was formed on the flask wall. The traces of solvents were further removed under N_2 stream. The lipid film was hydrated with ultrapure water or phosphate-buffered saline (PBS) to obtain DTX-loaded PDA liposome with a lipid concentration of 1 mg/mL. Then the solution was stirred at 50°C for 30 min, and the whole experiment was conducted in the dark. After that, the suspension was transferred into an autoclave and incubated by introducing CO_2 at 50°C and 20 MPa for 30 min. Finally, the CO_2 was released very slowly, and a transparent blank or drug-loaded PCDA liposome solution was obtained. The PCDA was polymerized under UV irradiation at 254 nm (24 W) within a photochemical chamber reactor and then heated to 70°C for 5 min. Then the solution was filtered through a 0.22-μm filter, cooled to room temperature, and kept at 4°C in the dark.

2: Dialysis method

Dialysis process is used to solve following problem:

1) Purify the PDA-liposome solution

   The acetone solution is found to have the ability to increase the solubility of free DTX and unpolymerized monomers of PDA. Acetone solution (5%, v/v) was used as dialysate, and a dialysis process was carried out and repeated many times until the concentration of free DTX in the PDA-liposome solution

was lower than 1% (determined using HPLC).

2) Determine the encapsulation of DTX in the liposomes
The free DTX in PDA-liposome solution should be measured to calculation the encapsulation of DTX in the liposome. The acetone solution (5%, v/v) was used as dialysate, and a dialysis process was carried out and repeated many times until the concentration of free DTX was lower than 1% (determined using HPLC).

3: Fluorescence cell imaging of DLPLs

The cancer cells were incubated with DLPLs for a certain time and then observed by the laser scanning confocal microscopy (LSCM) without washing. Seen from the dark fluorescence imaging, the cell body had shown a strong red fluorescence after 2 h, because the concentration of DLPLs internalized in cells was high. And if we magnified the image, there were some red fluorescence dots dispersed around the cancer cells. Thus, we deduced that almost all DLPLs were internalized by breast cancer cells.

4: Phase transition temperatures of DLPLs and DTX-loaded liposome.

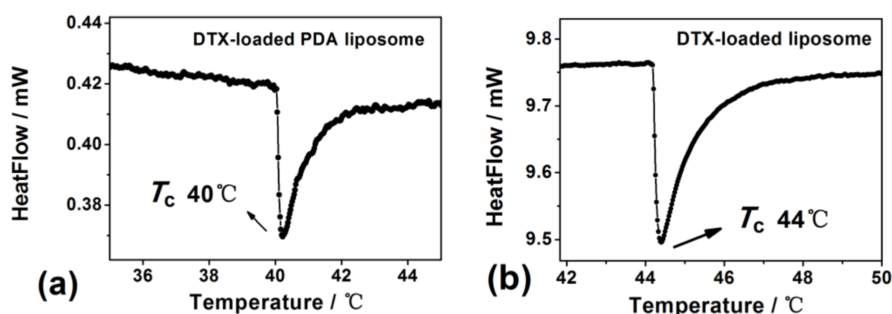

Fig. S2 Heating curves of (a) DLPLs and (b) DTX-loaded liposome measured by micro-DSC at a scanning rate of 1 °C / min.

5: Cell culture

Bcap-37 human breast cancer cells were grown in Dulbecco's modified Eagle's medium (DMEM) containing 10% (v/v) heat inactivated fetal bovine serum, 100 units/mL penicillin, and 100 units/mL streptomycin at 37°C in a moist atmosphere containing 5% CO_2. Exponentially growing Bcap-37 cells were exposed to drugs for specified time periods.

6: Cytotoxicity of PDA liposomes

To determine the cytotoxicity of PDA liposomes, the cell viability of Bcap-37

cells was evaluated by MTT (3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyltetrazolium bromide) assays and flow cytometry (FCM), as shown in Fig. S3.

MTT: Bcap-37 cells (10^4 cells/well) were placed in 96 well plates at 37°C in an atmosphere containing 5% CO_2. After overnight adherence, the cells were treated with fresh medium containing blank PDA liposome without DTX at a series of lipid concentrations, respectively. After 24 h, 20 μL of MTT reagent (0.5 mg/mL in PBS buffer solution) was added to each well. After incubation for 4 h, the upper medium was removed gently, and the formazan crystals were dissolved with 150 μL of dimethylsulfoxide (DMSO). The culture plates were shaken for 15 min in the dark to ensure the crystals were totally dissolved. The absorbance of each well was measured at 570 nm by an ELX808 IU Ultra Microplate Reader (Bio-Tek Instruments, Inc.). The spectrophotometer was calibrated to zero absorbance, using culture medium without cells. The relative cell viability (%) related to control wells was calculated by $[A]_{test}/[A]_{control}\times 100\%$. Where $[A]_{test}$ is the absorbance of the test sample, and $[A]_{control}$ is the absorbance of control sample. The cytotoxicity (%) was calculated as 100% minus the percentage of cell viability.

FCM: Apoptosis assays were performed by Annexin V-FITC/PI double staining and fluorescence-activated cell sorting (FACS) analysis. Bcap-37 cells from exponentially growing cultures (10^5 cells/mL) were seeded into 6 well plates. After overnight adherence, the cells were treated with blank PDA liposome separately. After 24 h, the cells were trypsinized and washed with phosphate-buffered saline (PBS). Annexin V-FITC/PI staining was performed in accordance with the manufacturer's protocol (BD Pharmingen, Franklin Lakes, NJ, USA). Then the samples were analyzed with a FACScan flow cytometry (FCM, Becton Dickinson & Co., USA). Meanwhile, apoptosis were analyzed using FlowJo software.

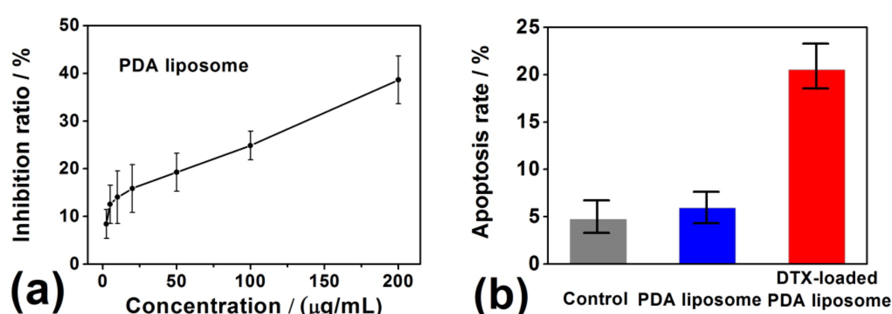

Fig. S3 (a) Inhibition ratios of PDA liposome at an increasing series of concentrations to Bcap-37 cells at 37°C for 24 h; (b) Apoptosis rates of Bcap-37 cells treated with PBS (grey column), bare PDA liposome (blue column) or DLPLs (red column) after incubation at 37°C for 24 h, respectively.