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Supporting information

CO_2 gas induced drug release from pH-sensitive liposome to circumvent doxorubicin resistant cells

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Materials

Hydrogenated soy bean phospholipids (HSPC), Cholesterol (Chol), and 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-n-[methoxy(polyethyleneglycol)-2000](mPEG$_{2000}$-DSPE) were purchased from the Avanti Polar Lipids, Inc. And all other reagents were of analytical grade without further purification.

Preparation of the doxorubicin-loaded liposomes

Briefly, liposome without drug was prepared by the lipid film method. A mixture of HSPC, Chol, and mPEG$_{2000}$-DSPE in a molar ratio of 62:33:5 was dissolved in chloroform and methanol (4:1, v:v), and evaporated dry to form the film at 40°C. Then, the liposome was produced by hydration of the lipid film with 300 mM NH_4HCO_3 solution at 50°C for 10min, and then size reduction with sonication, followed by replacing the external NH_4HCO_3 solution phase of the liposome by phosphate-buffed saline (PBS) using Sephadex G-50 column. Subsequently, the liposome was incubated with doxorubicin at 60°C for 10min and the novel liposome encapsulating doxorubicin (Lipo-c) was obtained. Another liposome loaded with doxorubicin (Lipo-s) was prepared by the same method described above; only $(NH_4)_2SO_4$ solution was used to hydrate the lipid film.

Cell culture

Human breast cancer cells sensitive (MCF-7S) and resistant (MCF-7R) to doxorubicin were maintained in Dulbecco's modified Eagle's medium (DMEM) and RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS) in a humidified atmosphere containing 5% CO_2 at 37°C. Single-cell suspensions were prepared by treatment with 0.25% (w/v) trypsin-EDTA.

Release study of doxorubicin-loaded liposomes with Lipo-s and Lipo-c

The release of doxorubicin from doxorubicin-loaded liposomes with Lipo-s and Lipo-c was determined by dialysis method using membrane with molecular weight cut-off of 10,000 Da. Briefly, the samples of Lipo-s and Lipo-c (0.5ml) were dialyzed against phosphate-buffed saline (PBS, pH7.4 and pH5.0) at 37°C. At the indicated time points (10min, 0.5h, 1h, 2h, 4h, 10h, 24h, 48h), 1ml aliquots of the medium were

withdrawn, and the same volume of fresh medium was added. The doxorubicin concentration was determined by fluorescence spectrometer. The accumulative release of doxorubicin from Lipo-s or Lipo-c was expressed as a percentage of the released doxorubicin and plotted as a function of time.

Cellular uptake study

The uptaken of the nanoparticles were investigated by the qualitative confocal image and the quantitative flow cytometry analysis. For confocal microscopic evaluation, MCF-7R cells were seeded onto 35-mm glass microscopy dish, and incubated at 37°C for 24 h. Then the culture medium was removed and the cells were incubated with free doxorubicin solution and doxorubicin-loaded liposomes diluted with medium of 10% FBS at a final concentration of 10μg/ml for 1h and 4 h at 37°C. After that, the cells were washed with PBS and then observed by confocal laser scanning microscopy (CLSM, Carl Zeiss, Germany) with excitation at 488 nm. For flow cytometry study, cells were seeded into 6-well plates at a density of 4×10^5 cells/well, then the doxorubicin and the liposomes were cultured with cells as described above. Finally, the cells were treated with trypsin-EDTA and collected, followed by the analysis of Attune® acoustic focusing cytometer (Applied Biosynthesis, Invitrogen, Germantown, MD). It was supported with the joint lab of nanotechnology for bioapplication, which was established with Life Technologies Inc in the National Center for Nanoscience and Technology of China.

Cytotoxicity

The cytotoxicity of doxorubicin-loaded liposomes against MCF-7S and MCF-7R cells were determined by (3-(4,5)-dimethylthiahiazo(-z-yl)-3,5-diphenytetrazoliumromide) (MTT) assay. Briefly, cells were seeded in a 96-well plate, and incubated for 24 h. Cells were then incubated with free doxorubicin, Lipo-s, and Lipo-c for 48h at various doxorubicin concentrations ranging from 0.08~50 μg/ml, or liposomes without doxorubicin from 0.016~1 mg/ml for 24h. Afterwards, the medium were replaced with 100 μl MTT at 0.5 mg/ml for 3.5 h, and then MTT solution were removed followed by the addition of 100 μl DMSO solution. At last, the absorbance was measured at 570 nm using microplate reader. Untreated cells in medium were used as control. All experiments were carried out with four replicates.

PCR

The reverse transcriptase-polymerase chain reaction (RT-PCR) was used for the analysis of mdr1 gene expression in the MCF-7S and MCF-7R cells according to the method manufacture's protocols. The primer sequences of mdr1 and GAPDH was as follows:

mdr1 forward of 5'-GTCCCAGGAGCCCATCCT-3'

and reverse of 5'-CCCGGCTGTTGTCTCCATA-3';

GAPDH forward of 5'-GACTTCAACAGCAACTCCCAC-3'

and reverse of 5'-TCCACCACCCTGTTGCTGTA-3'.

RT-PCR parameters were as follows: 50°C for 2min, 10min of Taq activation at 95°C, followed by 45 cycles of PCR at 95°C×15s, 60°C×1min. Specificity was demonstrated by melt curve analysis and agarose gel electrophoresis in Fig. S2.

Statistical analysis

All experiments were carried out in triplicate unless otherwise indicated. Error bars represent standard deviations. Data are presented as mean value ± SD from three independent measurements.

Figure S1.

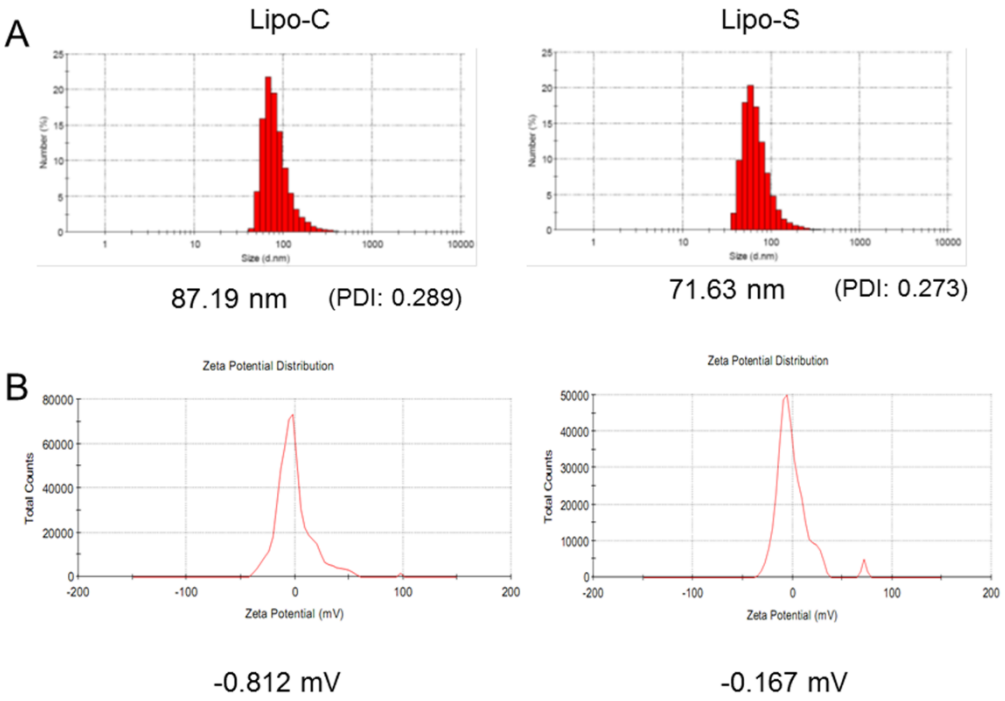

Figure S1. Size and Zeta-potential of the Lipo-s and Lipo-c nanoparticles.

Figure S2.

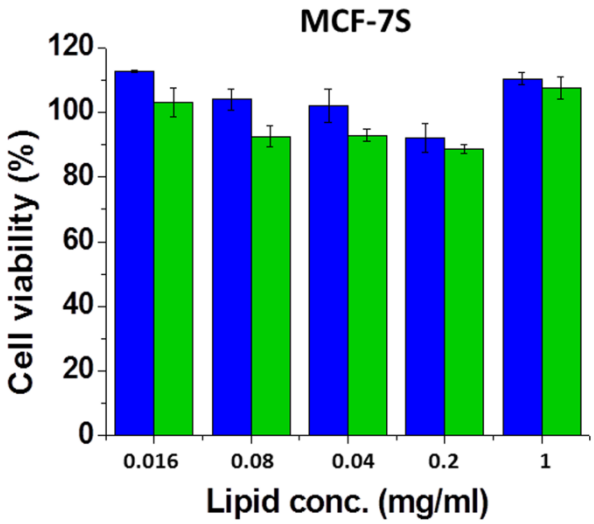

Figure S2. Cell viability of MCF-7S after treatment with the blank liposomes.

Figure S3.

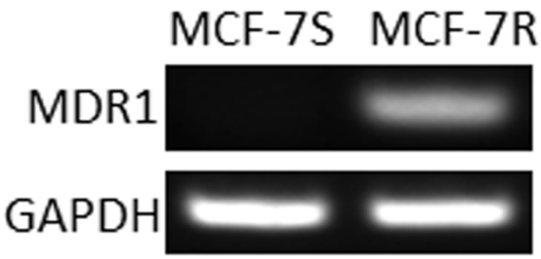

Figure S3. The MDR1 gene expression in the sensitive (MCF-7S) and resistant (MCF-7R) cells to doxorubicin determined by PCR. Images were observed by agarose electrophoresis.