Supporting Information

Phosphorylcholine zwitterionic shell-detachable mixed micelles for enhanced cancerous cellular uptakes and increased DOX release

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Materials

2-Methacryloyloxyethyl phosphorylcholine (MPC, Nanjing Joy-Nature Science & Technology Development Institute, China), stannous octoate, $Sn(Oct)_2$ (Sigma-Aldrich), ε-caprolactone (εCL, Sigma-Aldrich), 2,2-azoisobutyronitrile (AIBN, Shanghai Covalent Chemical Technology Co.), doxorubicin hydrochloride (DOX·HCl, Chengdu Aisite Chemical Technology Co., Ltd.) were used as-received. 2-bromoisobutyryl bromide and 2-hydroxydisulfide were both purchased from admas. Toluene and triethylamine (TEA) were dried over sodium or CaH_2 respectively, and then distilled prior to use. Mouse connective tissue fibroblast cells (L929), human cervical carcinoma cells (HeLa) and human breast adenocarcinoma cells (MCF-7) were obtained from Saiqi (Shanghai) Biological Engineering Co., Ltd. Dulbecco's modified Eagle medium (DMEM), fetal bovine serum (FBS), penicillin-streptomycin antibiotic solution, 0.25% trypsin ethylenediaminetetraacetic acid solution, and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetra zolium bromide (MTT) were obtained from HyClone. In addition, all the other reagents were used without further purification.

Measurements

Molecular weights were characterized on an Agilent 1260 Infinity II series gel permeation chromatograph (GPC), which equipped with a refractive index detector and PL gel 10 μm MIXED-B columns. A series of narrow polystyrene (PS) were used as standards (Agilent Technologies) for the calibration. THF was applied as the eluent at a flowing rate of 1.0 mL min^{-1} under 35 °C. Nuclear Magnetic Resonance (NMR) spectra were recorded on Varian INOVA 400 MHz spectrometers. Tetramethylsilane (TMS) was applied as an internal chemical shift reference. Fourier Transform Infrared (FT-IR) spectra were recorded on a Bruker Equinox 55 spectrometer via the KBr pellet technique averaged with 32 accumulations at a resolution of 4.0 cm^{-1}.

The critical micelle concentration (CMC) was determined using pyrene as a fluorescence probe. The CMC was estimated as the cross-point extrapolating the intensity ratio of I_{339} and I_{333}.

The average size, size distribution, and zeta-potential of the micelles were measured with dynamic light scattering (DLS) using a Malvern Zetasizer Nano ZS90 instrument. The stability of polymeric micelles under various conditions was tested by DLS through monitoring of the particle size change as a function of time.

Test conditions: in pure water, phosphate buffered saline (PBS, pH 7.4) solution and DMEM medium at room temperature for 50 h.

Water contact angles (WCA) of the micelle suspensions coated surfaces were recorded with a video-based contact angle goniometer (DSA25, KRüSS, Germany). 2 μL deionized water was dropped on the prepared surfaces and the WCA values were calculated by averaging at least five data at different locations. The WCA values were expressed as means±SD.

The drug loading content (LC), encapsulation efficacy (EE) and cumulative drug release percent (E_r) were assessed by ultraviolet spectroscopy method and calculated by equations 1~3, respectively.

$$\mathrm{LC(wt\%)} = \frac{m_{\text{DOX in the micelles}}}{m_{\text{DOX loaded micelles}}} \times 100 \qquad (1)$$

$$\mathrm{EE(wt\%)} = \frac{m_{\text{DOX in the micelles}}}{m_{\text{DOX in feed}}} \times 100 \qquad (2)$$

$$E_r\mathrm{(wt\%)} = \frac{V_e \sum_1^{n-1} C_i + V_0 C_n}{m_{\text{DOX in the micelles}}} \times 100 \qquad (3)$$

Where $m_{\text{DOX in the micelles}}$, $m_{\text{DOX loaded micelles}}$ and $m_{\text{DOX in feed}}$ represent the mass of DOX in micelles, the mass of DOX loaded micelles and the mass of DOX in feed, respectively. And in equations 3, C_n and C_i (mg·mL^{-1}) are the concentration of the dialysate withdrawn at the interval of t_n and t_i, respectively. V_e (4.0 mL) and V_0 (20.0 mL) are the volumes withdrawn at the interval and the total volume of the release medium, respectively.

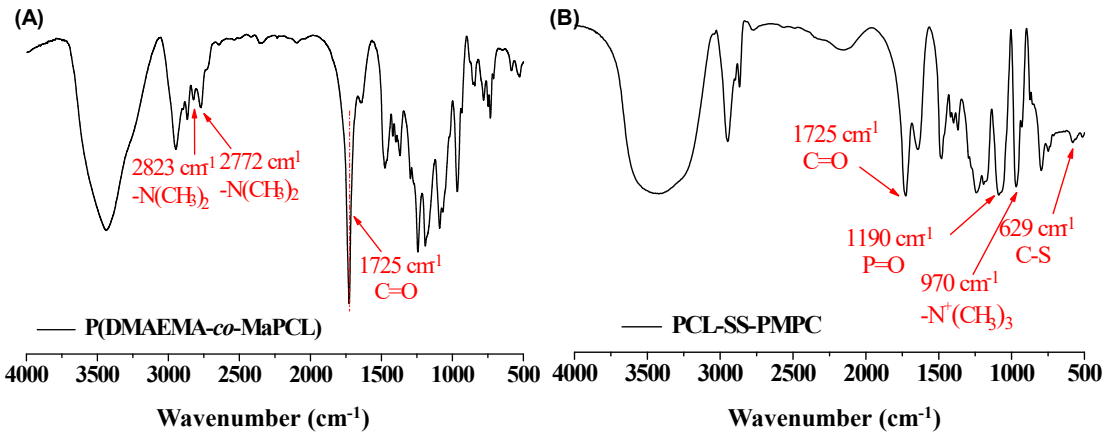


Fig. 1 FT-IR spectra of the P(DMAEMA-*co*-MaPCL) (A) and PCL-SS-PMPC (B).

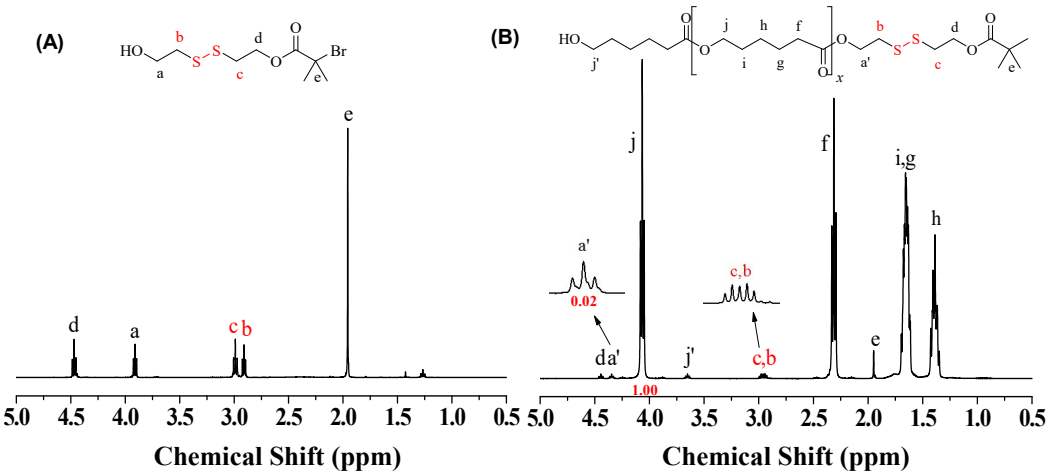
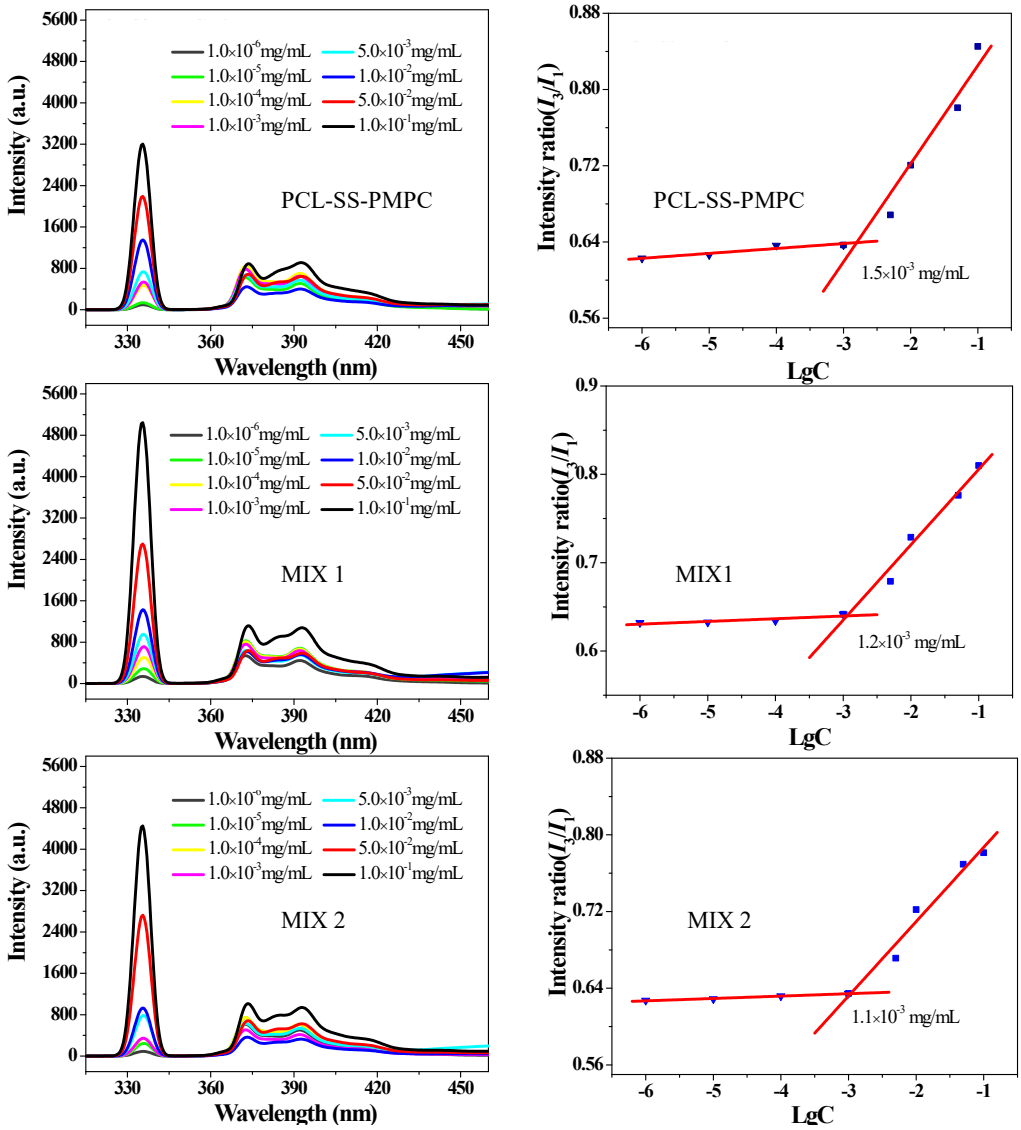


Fig. 2 ^{1}H NMR spectra of HO-SS-^{i}BuBr (A) and PCL-SS-^{i}BuBr (B) in $CDCl_3$.



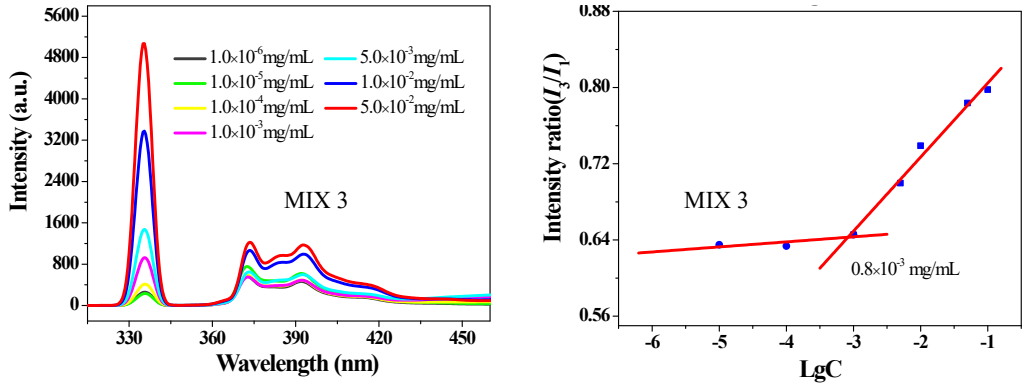


Fig. 3 Fluorescence emission spectrum of pyrene in PCL-SS-PMPC and MIX micelles. Plots of I_3/I_1 as a function of polymer concentrations.

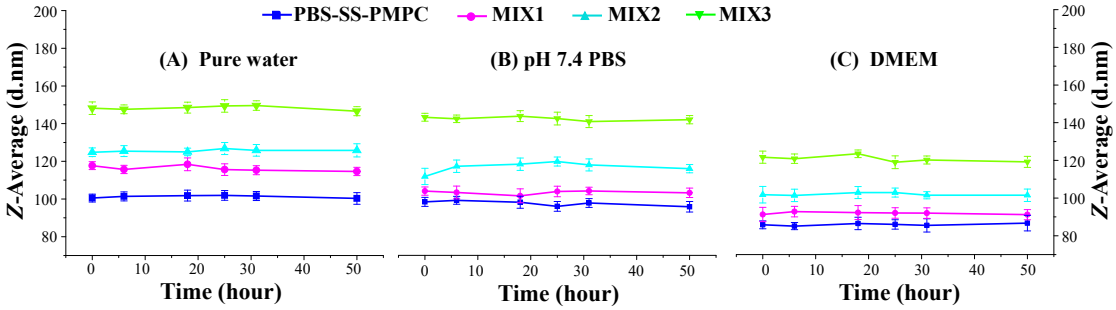


Fig. 4 Size changes of blank PCL-SS-PMPC, MIX 1, MIX 2 and MIX 3 micelles in pure water (A), pH 7.4 PBS (B) and DMEM (C) within 50 h. The concentrations of the micelles were 0.5 mg mL^{-1}.

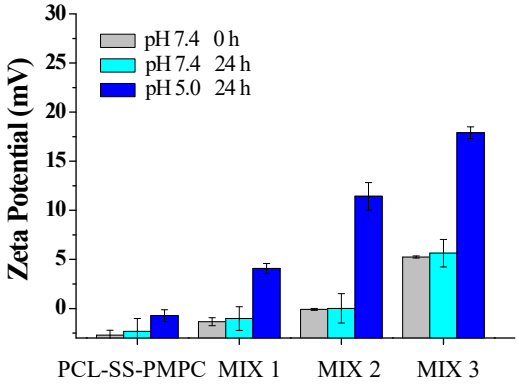


Fig. 5 Zeta potential of micelles at pH 7.4 and pH 5.0.

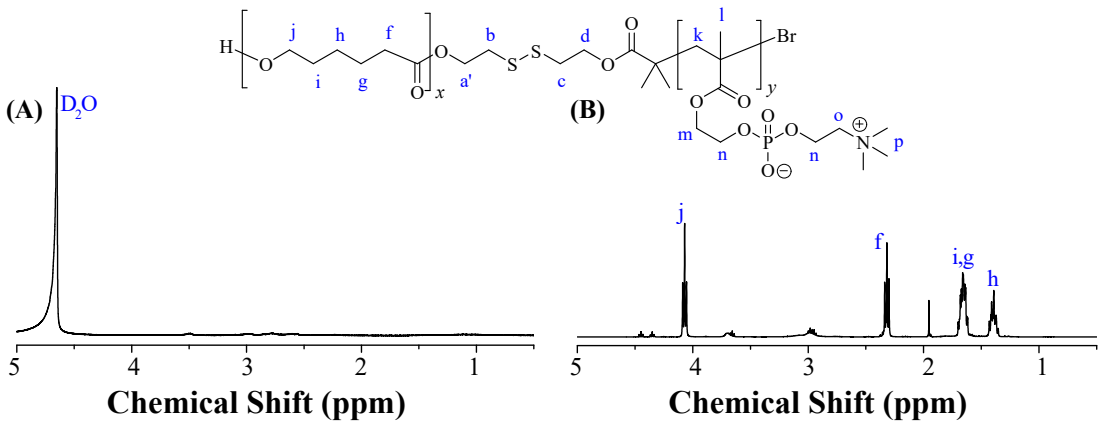


Fig. 6 ^{1}H NMR spectra of the freeze-dried powder of PCL-SS-PMPC after DTT treatment in D_2O (A) and in $CDCl_3$ (B).

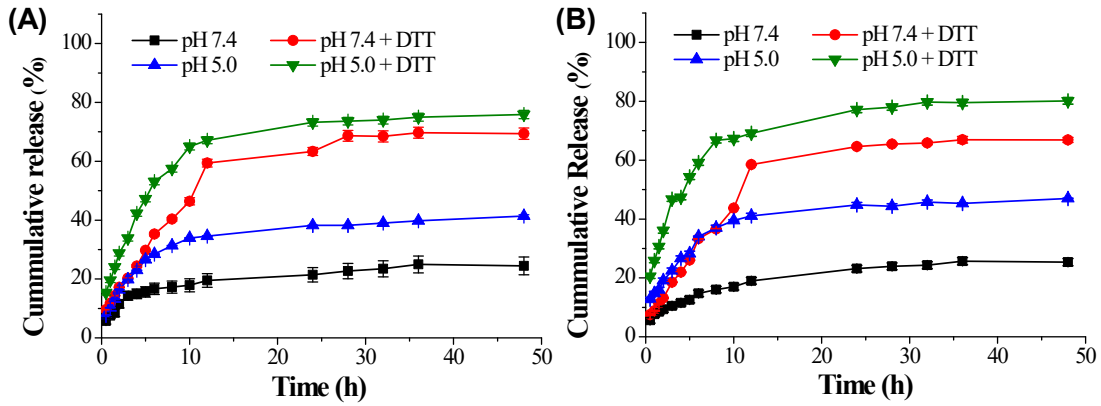


Fig. 7 *In vitro* DOX release of DOX-loaded MIX 1 (A) and MIX 2 (B) micelles under different conditions: pH 7.4 and pH 5.0 with or without 10 mM DTT.

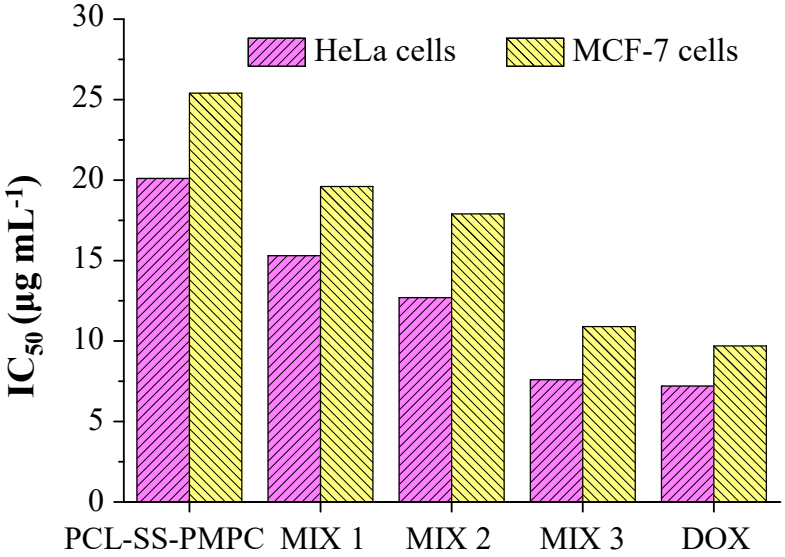


Fig. 8 IC_{50} values of DOX-loaded micelles and free DOX against HeLa and MCF-7 cells.

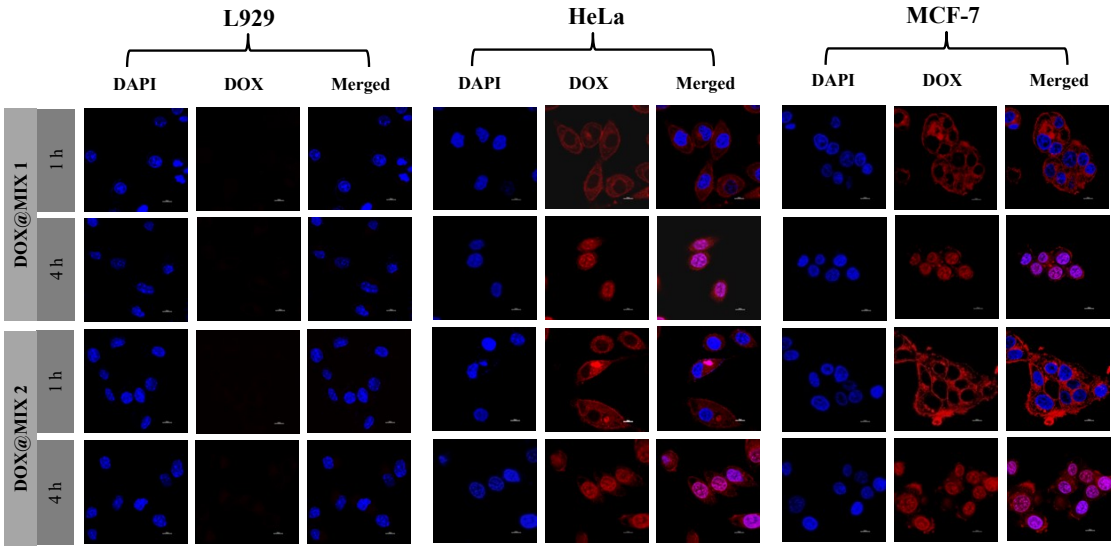


Fig. 9 CLSM images of L929, HeLa and MCF-7 cells incubated with the DOX@ MIX 1 and DOX@MIX 2 for 1 h and 4 h. The nuclei were stained blue with DAPI, and the red color was DOX fluorescence. The DOX concentration was 10 μg mL^{-1}.

Table 1 Synthesis and characterization of polymer P(DMAEMA-*co*-MaPCL).

Polymer	Molar ratio of comonomers[a]	$M_{n,NMR}$ [a]	$M_{n,GPC}$ [c]	PDI [c]
P(DMAEMA-*co*-MaPCL)	12:1 [b]	- [d]	11,000	2.97

[a]Calculated by the ^{1}H NMR spectrum; [b]Molar ratio of comonomers of DMAEMA and MaPCL based on the formula $m/n=14I_r/I_h$ (Fig. 1A, DP of the PCL in MaPCL is 28). [c]Measured by GPC using DMF as eluent. [d]Can not be calculated.

Table 2 Synthesis and characterization of polymer PCL-SS-PMPC.

Polymers	[CL] [a]	[MPC] [a]	$M_{n,NMR}$ [a]	$M_{n,GPC}$ [b]	PDI [b]
PCL-SS-^{i}BuBr	50	-[c]	6,000	6300	1.37
PCL-SS-PMPC	50	51	21,000	- [c]	- [c]

[a]Calculated by the ^{1}H NMR spectrum; [b]Measured by GPC using THF as eluent. [c]Not calculated or measured.

Table 3 Characterization of blank micelle and DOX-loaded micelle.

Sample[a]	CMC (mg/mL)	Blank micelles			DOX-loaded micelles				
		Size (d.nm)	PDI	ζ-Potential (mV)	Size (d.nm)	PDI	ζ-Potential (mV)	LC (%)	EE (%)
PCL-SS-PMPC	1.5×10^{-3}	91.5±1.3	0.195	-4.7±1.9	101.1±1.0	0.213	0.2±2.1	7.5	30.8
MIX 1	1.2×10^{-3}	99.4±0.9	0.212	0.1±0.8	116.9±1.2	0.222	8.9±1.1	9.1	38.7
MIX 2	1.1×10^{-3}	121.8±1.5	0.215	4.6±1.3	147.8±1.8	0.189	14.8±0.9	9.7	42.6
MIX 3	0.8×10^{-3}	136.2±2.0	0.221	12.8±1.1	165.5±2.1	0.198	22.3±1.6	11.4	49.9

A represents PCL-SS-PMPC; B represents P(DMAEMA-*co*-MaPCL); [a]Mass ratio of polymer A and B