Supporting Information

Folate-Conjugated Cell Membrane Mimetic Polymer Micelles for Tumor-Cell-Targeted Delivery of Doxorubicin

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Electronic supplementary information (ESI) available:

Experimental details of copolymer of P(MPC-*co*-MaPCL) and macromonomer of FAMEG; ^{1}H NMR and FT-IR spectra of related monomers and copolymers; CMC plots, size distributions in different media and DSC curves of as-prepared copolymers; cell viability and cellular uptake assay of copolymer micelles.

Synthesis of macromonomer, MaPCL. MaPCL was synthesized through ring-opening polymerization (ROP) of ε-CL according to our previous report [*Polym. Chem.* **2016**, 7, 5698-5708]. And the synthetic route was shown in **Scheme S1**. Briefly, 0.3 g of HEMA (2.3 mmol), 7.9 g of ε-CL (69.0 mmol), 0.1 g of $SnOct_2$ and 20 mL of dry toluene were added to a flame-dried three-necked flask with a stopcock. Then, the flask was heated on an oil bath at 130 ^{o}C with vigorous stirring for 24 h under a N_2 atmosphere. After evaporation of the solvent, the resulting product was dissolved in $CHCl_3$ and precipitated three times with an excess amount of cold CH_3OH. The white powder MaPCL was collected and dried in a vacuum oven at room temperature for about 24 h until a constant weight. And the yield was about 72%.

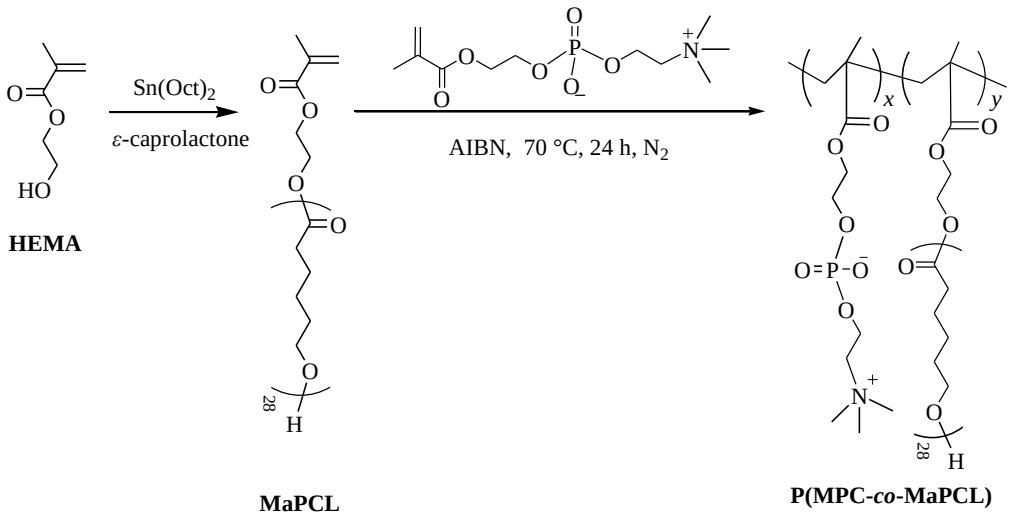
The ^{1}H NMR of MaPCL was shown in **Figure S1a**. The degree of polymerization (n) of CL in MaPCL was calculated from the ^{1}H NMR spectrum according to **Equation S1**. The average molecular weight (M_n, NMR) of MaPCL was calculated by **Equation S2**.

$$n = \frac{I_{2.31}}{2I_{6.13}} \qquad \text{(S1)}$$

$$M_n = 129 + 114\ n \qquad \text{(S2)}$$

where n is the degree of polymerization of ε-CL. 114 and 129 are the molecular weight of ε-CL and HEMA, respectively. $I_{2.31}$ and $I_{6.13}$ are the relative intensities of the peaks at 2.31 (-(O)CC$\boldsymbol{H}_2$-, 2nH) and 6.13 (-C(CH_3)=C$\boldsymbol{H}_2$, 1H) ppm.

The degree of polymerization (n) of CL in MaPCL was calculated to be 28. The average molecular weight ($M_{n,NMR}$) of MaPCL was calculated to be 3321 g mol^{-1}.



Scheme S1 The synthetic routes of macromonomer, MaPCL and copolymer of P(MPC-*co*-MaPCL).

Synthesis of copolymer of P(MPC-*co*-MaPCL). Copolymer of P(MPC-*co*-MaPCL) was synthesized using free radical polymerization based on our previous report [*Polym. Chem.* **2016**, 7, 5698-5708]. And the synthetic route was shown in **Scheme S1**. 0.3 g of MaPCL (0.09 mmol) and 0.3 g of MPC (1.01 mmol) were dissolved in 30 mL of EtOH and THF. And 0.023 g of AIBN (0.14 mmol) was dissolved in 8 mL of THF. The flame-dried three-necked flask containing 10mL EtOH and one fourth of the AIBN solution was sealed and purged with nitrogen for 30 min and then heated under stirring at 73 ^{o}C, and then dropwise monomer solution above prepared into the flask. Copolymers were obtained by dissolving (in $CHCl_3$) precipitated (in cold *n*-hexane) method. The product was dried in a vacuum oven at 40 ^{o}C for 24 h. And the yield was approximately 80%.

The ^{1}H NMR of MaPCL was shown in **Figure S1b**. The molar ratio of MPC and MaPCL units (*x* : *y*) in the copolymer was determined by the ^{1}H NMR spectrum of P(MPC-*co*-MaPCL) according to **Equation S3**.

$$\frac{x}{y} = 2n \times \frac{I_{3.30}}{9I_{2.31}} \qquad (S3)$$

where *n* stands for the degree of polymerization of ε-CL and is calculated to be 28 based on **Equation S1,** and $I_{3.30}$ and $I_{2.31}$ are the relative intensities of the peaks at 3.30 ppm (MPC segment, 9 H) and 2.31 ppm (MaPCL segment, 2n H).

The molar ratio (*x*:*y*) of PMPC and PMaPCL was 9:1 in the copolymer of P(MPC-*co*-MaPCL).

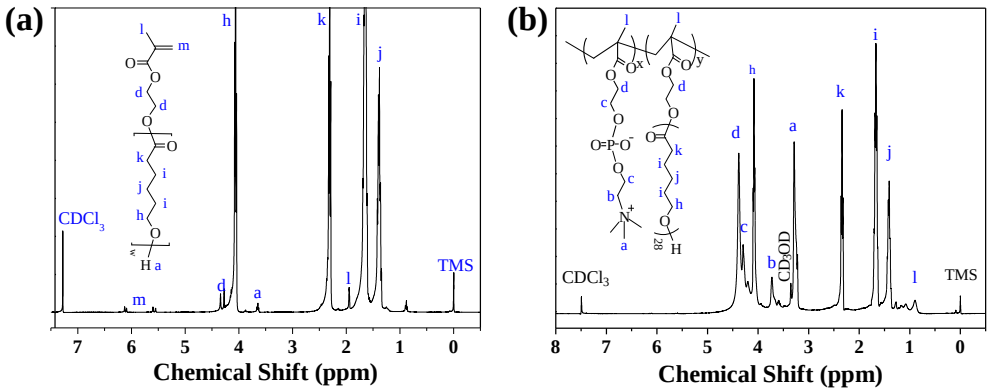
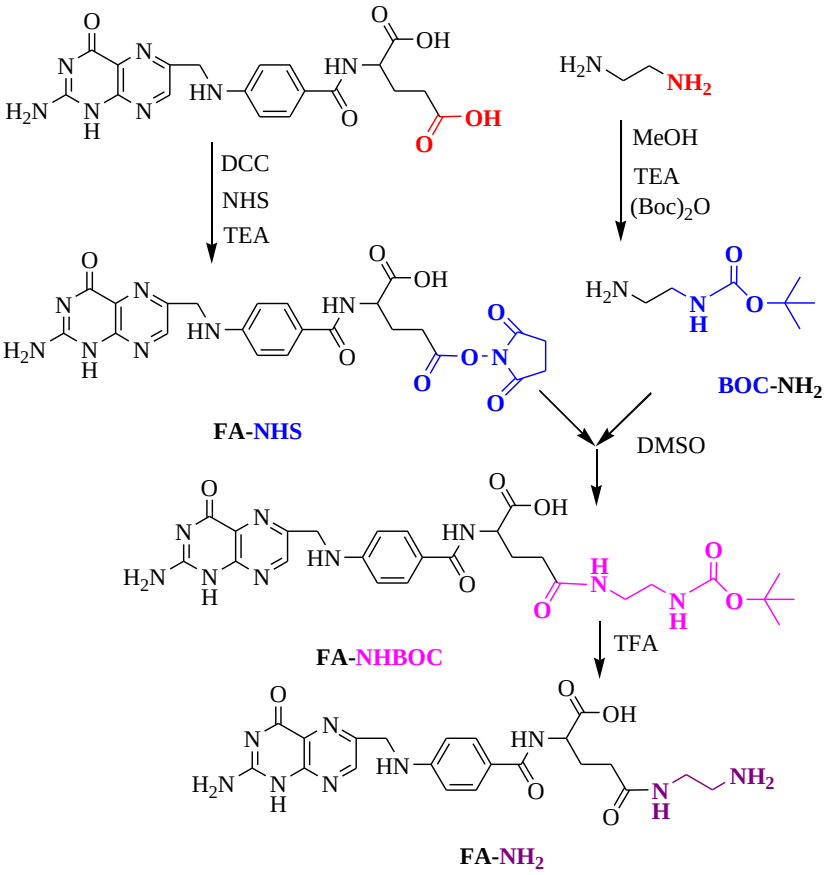


Figure S1 ^{1}H NMR spectra of MaPCL in $CDCl_3$ and P(MPC-*co*-MaPCL) in the mixture solution of $CDCl_3$ and CD_3OD (v/v=1:1)

Synthesis of FA-NH$_2$. FA-NH$_2$ was synthesized via a four-step synthetic strategy [*J. Mater. Chem. B* **2016**, 4, 5464-5474]. Firstly, FA and NHS reacted in the presence of DCC at room temperature for 24 h to obtain FA-NHS solution. Secondly, an oily product of monoamino-protected ethylenediamine (BOC-NH$_2$) was prepared using $(Boc)_2O$ and EDA as raw materials in the mixed solution of CH_3OH and TEA (*v*/*v* = 10:1) at room temperature for 10 h. Then, monoamino-protected folic acid (FA-NHBOC) was prepared by adding BOC-NH$_2$ into the above FA-NHS solution in the dark and stirred at room temperature. Finally, FA-NH$_2$ was obtained as a yellow powder after the reaction of FA-NHBOC and trifluoroacetic acid at room temperature for 2 h.



Scheme S2 The synthetic routes of FA-NH_2.

The chemical structure of FA-NH_2 was confirmed by ^{1}H NMR as shown in **Figure S2**. Peaks appeared at 2.90 and 3.31 ppm assigned to two methylene hydrogen of the ethylene diamine segment [*Polymer* **2011**, 52, 987-995; *Chem. Commun.* **2010**, 46, 2632-2634], and both the peak areas are almost equal. These evidences indicated that FA-NH2 was successfully prepared by the above proposed synthesis procedures.

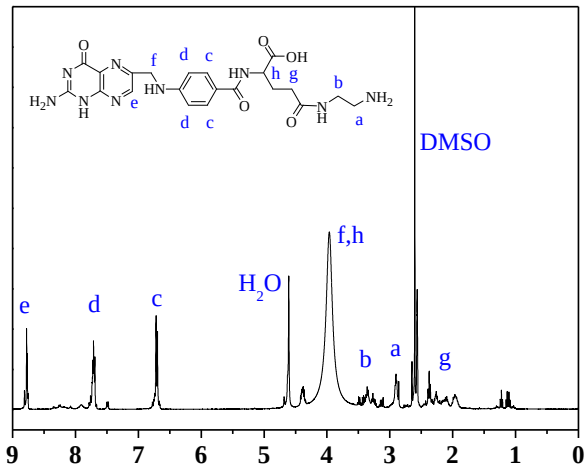
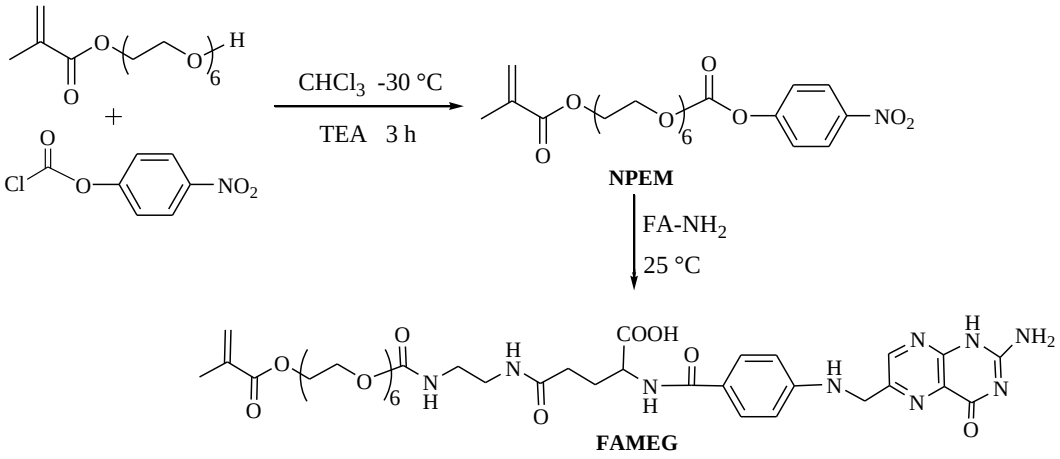


Figure S2 ^{1}H NMR spectrum of FA-NH_2 in the mixed solvent of $CDCl_3$ and DMSO (d_6).

Synthesis of FAMEG. The monomer, FAPEG was synthesized by a two-step synthetic strategy shown as **Scheme S3**. 14.4 g of polyethylene glycol methacrylate (M_n≈360, 0.04 mol), 4.0 g of TEA (0.04 mol) and 60 mL of $CHCl_3$ were added in a 250 mL three-neck flask. After the solution was cooled to -30 ºC, 10.1 g of *p*-nitrobenzoyl chloride (NPC, 0.05 mol) in 60 mL $CHCl_3$ was added dropwise. And the reaction mixture was maintained at -30 °C for 4 h and then allowed to react at room temperature overnight. The precipitate was filtered off and the filtrate was concentrated under reduced pressure. NPEM was obtained as a yellow oily liquid by precipitation in diethyl ether. Then, FAMEG was synthesized by the amidation of the active ester groups in NPEM with FA-NH_2 in DMSO at 80 ºC for 24 h in the dark. The chemical structures of NPEM and FAMEG was confirmed by the ^{1}H NMR spectra shown in **Figure S3**.



Scheme S3 The synthetic routes of NPEM and FAMEG.

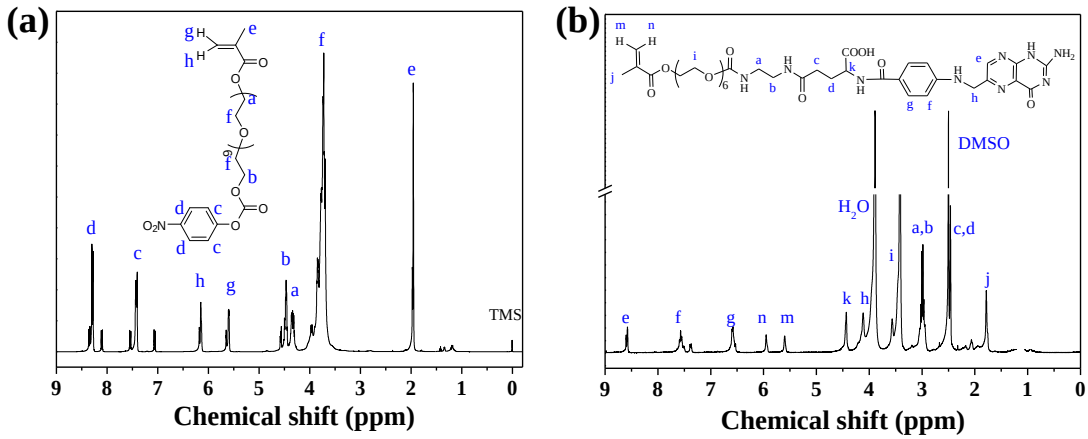


Figure S3 ^{1}H NMR spectra of NPEM in $CDCl_3$ and FA-NH_2 in DMSO (d_6).

$$\mathrm{LC\,(wt\,\%)} = \frac{\text{weight of DOX in the micelles}}{\text{weight of DOX loaded micelles}} \times 100\% \qquad \text{(S4)}$$

$$\mathrm{EE\,(wt\,\%)} = \frac{\text{weight of DOX in the micelles}}{\text{weight of DOX in feed}} \times 100\% \qquad \text{(S5)}$$

$$\mathrm{Abs} = 18.368\mathrm{C} + 0.0307 \qquad R^2 = 0.9919 \qquad \text{(S6)}$$

$$\mathrm{E_r} = \frac{\mathrm{V_e}\sum_1^{n-1}\mathrm{C_i} + \mathrm{C_i V_0}}{\mathrm{m_{drug}}} \qquad \text{(S7)}$$

$$\mathrm{Abs} = 17.225\mathrm{C} + 0.0166 \qquad R^2 = 0.9951 \qquad \text{(S8)}$$

$$\mathrm{Abs} = 17.286\mathrm{C} + 0.0145 \qquad R^2 = 0.9987 \qquad \text{(S9)}$$

$$\text{Cell Viability}\ (\%) = \frac{\mathrm{OD_{sample}}}{\mathrm{OD_{control}}} \times 100\% \qquad \text{(S10)}$$

where LC and EE are the drug-loading content and encapsulation efficiency, respectively; Abs is the absorbance and C is the concentration (mg mL^{-1}); E_r stands for the accumulative release rates; V_0 is the total volume of the release medium (25 mL); C_n (mg L^{-1}) is the

concentration of the sample withdrawn at the interval of t_n; V_e and C_i are the volume and concentration of sample withdrawn at the interval of t_i, respectively; and m_{drug} (μg) is the total amount of DOX in the micelles; OD is the optical density at 490 nm in each well.

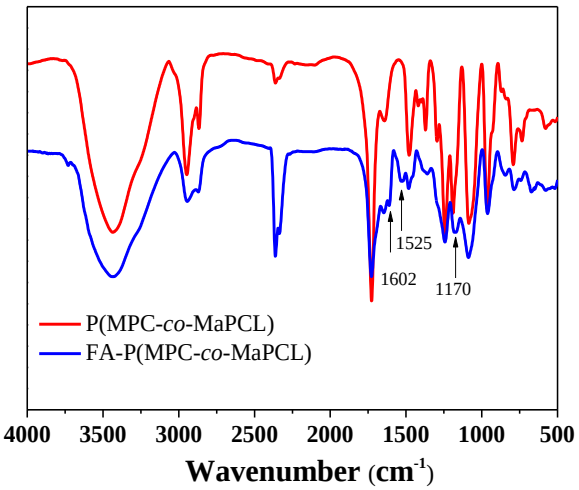


Figure S4 FT-IR spectra of copolymers of P(MPC-*co*-MaPCL) and FA-P(MPC-*co*-MaPCL).

Critical micelle concentration (CMC). The CMCs of the copolymers P(MPC-*co*-MaPCL) and FA-P(MPC-*co*-MaPCL) were determined using pyrene as a fluorescence probe.[*ACS Appl. Mater. Interfaces* **2016**, 8, 17109−17117] 100 μL of benzene solution of pyrene (5.0×10^{-5} mol L^{-1}) was pipetted into a series of volumetric flasks (10 mL) and the benzene was evaporated at room temperature. And then, copolymer solutions with various concentrations were added to each of the flask. The solutions were equilibrated at room temperature for 1 day in dark. The fluorescence intensity was recorded on an F-2700 fluorescence spectrophotometer reader at λ_{ex} = 335 nm. The ratios of fluorescence intensities at 384 nm over 373 nm (I_{384}/I_{373}) were plotted against the copolymer concentration. The value of the intersection of two tangents was considered as the CMC.

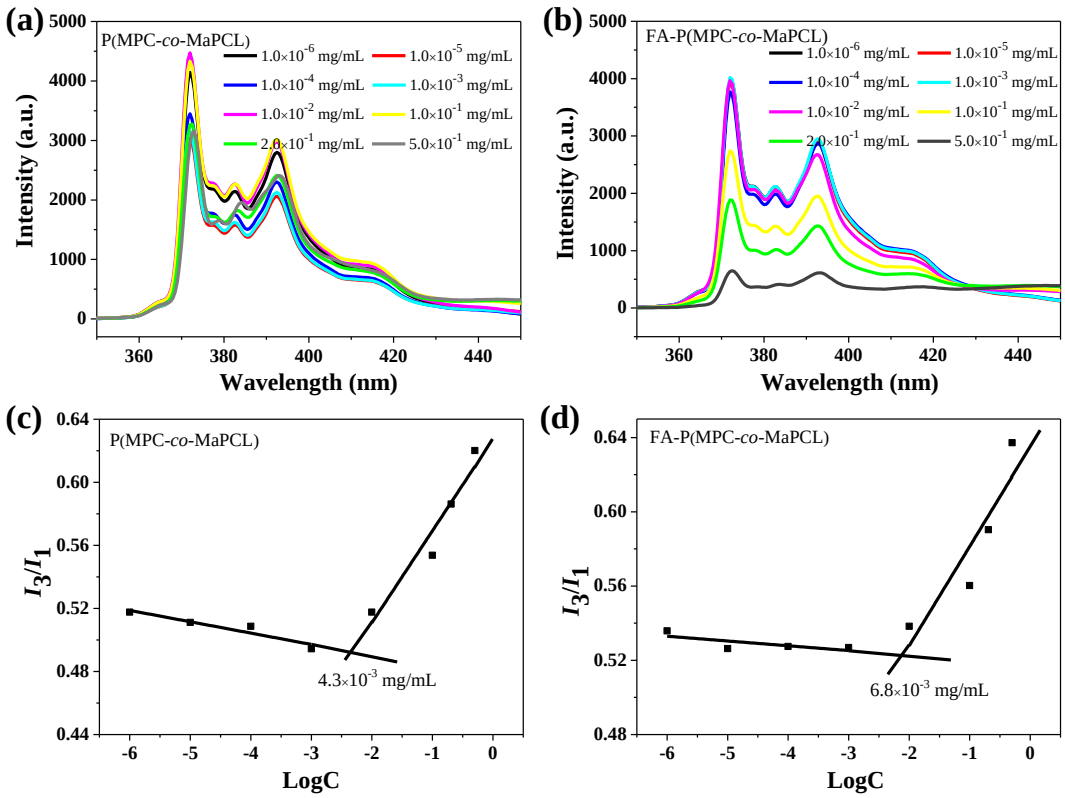


Figure S5. Fluorescence emission spectrum of pyrene in P(MPC-*co*-MaPCL) (a) and FA-P(MPC-*co*-MaPCL) (b) micelles (excitation wavelength of 335 nm). The intensity ratio (I_3/I_1) of the pyrene emission versus the log concentration of P(MPC-*co*-MaPCL) (c) and FA-P(MPC-*co*-MaPCL) (d) micelles.

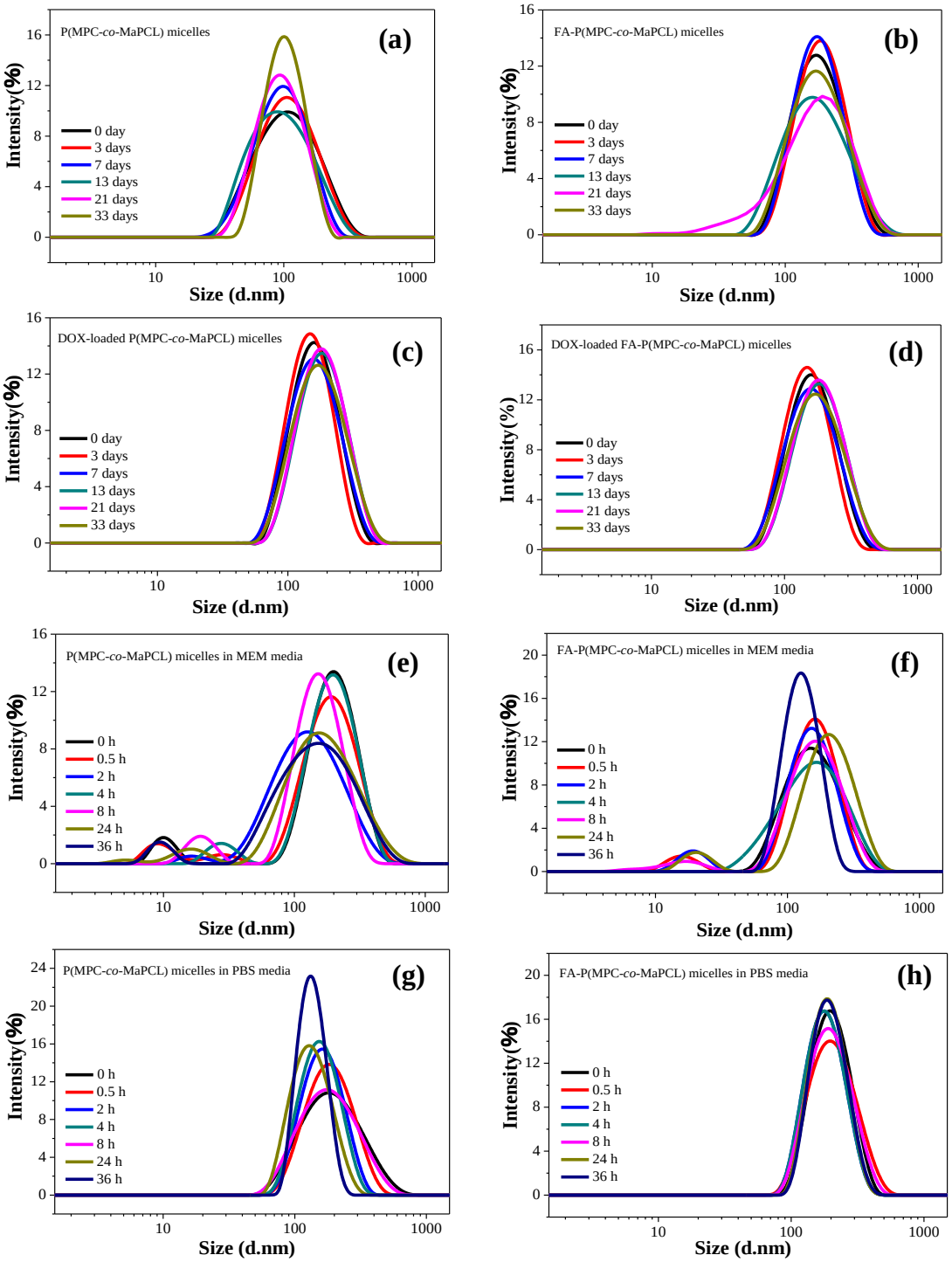


Figure S6. The changes of size distributions of P(MPC-*co*-MaPCL) (a, c) and FA-P(MPC-*co*-MaPCL) (b, d) micelles in pure water (Concentration = 1.0 mg mL^{-1}) within 33 days before and after DOX-loaded, and in MEM (e, f) and PBS (pH 7.4) (g, h, Concentration = 0.5 mg mL^{-1}) within 30 hours by DLS measurements.

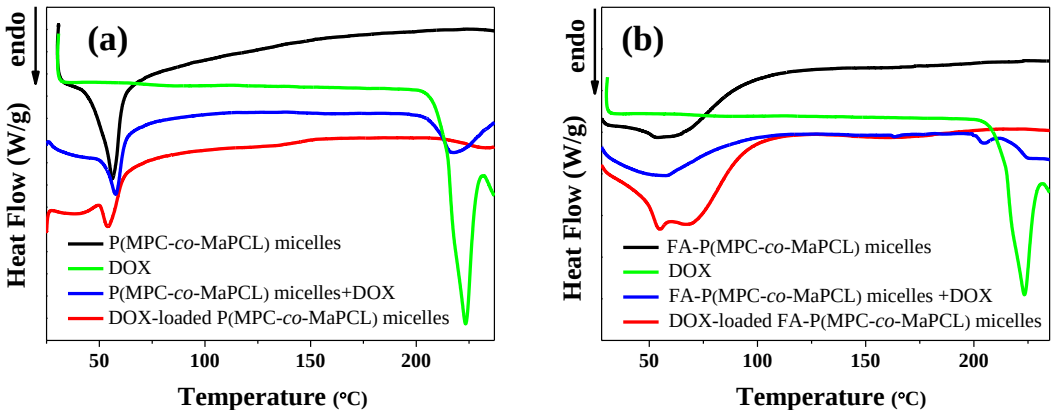


Figure S7. DSC thermograms of the first heating scan recorded at 10 °C/min, blank P(MPC-*co*-MaPCL) and FA-P(MPC-*co*-MaPCL) micelles (black line), free DOX (green line), physical mixture of free DOX and P(MPC-*co*-MaPCL) or FA-P(MPC-*co*-MaPCL) micelles (blue line), and DOX-loaded P(MPC-*co*-MaPCL) or FA-P(MPC-*co*-MaPCL) micelles (red line).

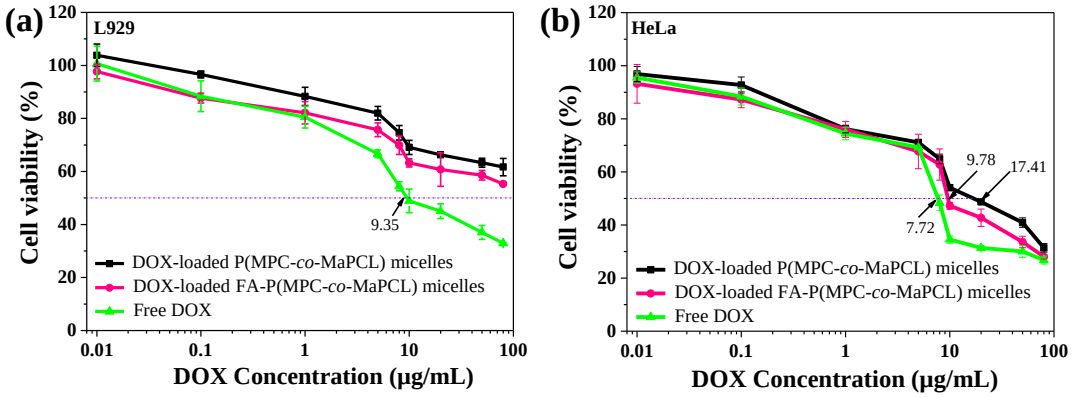


Figure S8. Cell viability of L929 cells (a) and HeLa cells (b) against the DOX-loaded polymeric micelles and free DOX after incubation at different concentrations for 24 h.

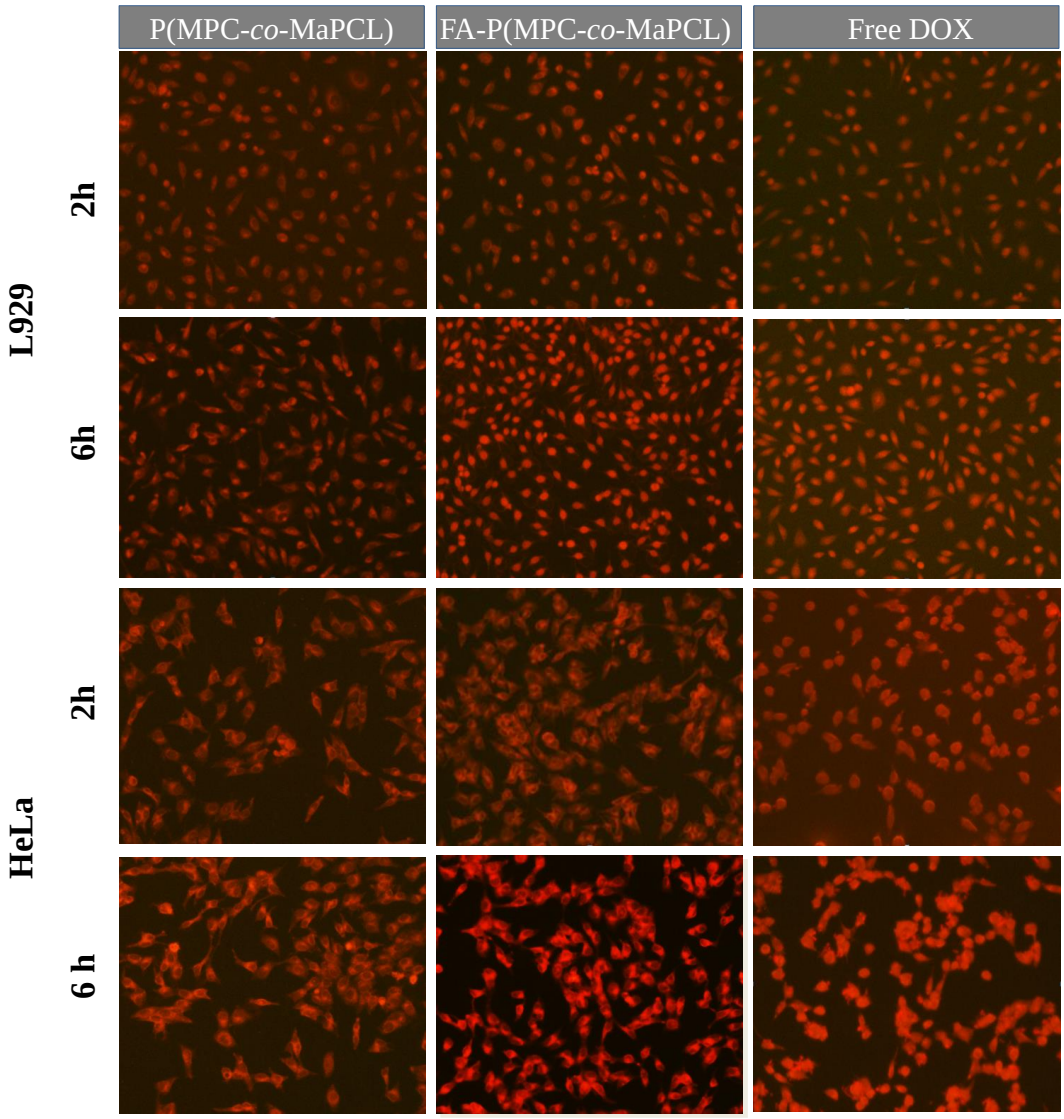


Figure S9**.** Fluorescence microscopic images of L929 and HeLa cells incubated with DOX loaded polymeric micelles and free DOX at 37 ^{o}C for 2 h and 6 h. DOX concentration was 10 μg mL^{-1}.