

Fabrication of micelles from poly(butylene succinate) and poly(2-methacryloyloxyethyl phosphorylcholine) copolymers as potential drug carrier

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Abstract

A series of copolymers of poly(2-methacryloyloxyethyl phosphorylcholine)-*b*-poly(butylene succinate)-*b*-poly(2-methacryloyloxyethyl phosphorylcholine) [PMPC-*b*-PBS-*b*-PMPC] were synthesized by atom transfer radical polymerization (ATRP). The structure of the polymers were characterized by proton nuclear magnetic resonance spectroscopy (¹H NMR) and infrared spectroscopy (IR), and their thermal properties were described using thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC). In aqueous solutions, the PMPC-*b*-PBS-*b*-PMPC could form micelles with sizes ranging from 108 to 170 nm. The *in vitro* release studies showed that acidic media and longer PMPC chain benefited DOX release. MTT assays indicated the

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micelles had low cytotoxicity to Hela and L929 cells. DOX-loaded micelles exhibited high cytotoxicity to Hela cells. Flow cytometry results demonstrated that DOX-loaded micelles could be internalized by Hela cells. The *in vitro* phagocytosis results showed 3.9-fold and 5.5-fold reductions compared with PLA nanoparticles and PDS55 micelles. These results demonstrate that PMPC-*b*-PBS-*b*-PMPC block copolymer micelles have great promise for cancer therapy.

Keywords: poly(butylene succinate), poly(2-methacryloyloxyethyl phosphorylcholine), ATRP, micelles, drug carrier

INTRODUCTION

In recent years, polymeric micelles have been extensively studied as one of the most promising drug delivery system (DDS) to achieve improved cancer chemotherapy.^{1,2} Micelles with the size of 20~200 nm may preferentially be accumulated in the tumor tissue through the enhanced permeability and retention (EPR) effect.³ Moreover, micelles demonstrate many superior features, including reduced side effects, improved drug tolerance and drug bioavailability.⁴⁻⁶ Nevertheless, one practical issue with conventional drug-loaded polymeric micelles is that they can be recognized and entrapped by the monocyte phagocytic system (MPS), and then quickly eliminated following intravenous administration, resulting in short circulation lifetime and intolerable toxicity.⁷⁻⁹ Thus, the long circulation of micelles is an important prerequisite, which can ensure the encapsulated drug be delivered to the tumor site.

In the micelle systems, poly(ethylene glycol) (PEG) is the most widely-used hydrophilic modifier, because it is approved by the US Food and Drug Administration (FDA) owing to its strong hydration ability, conformational flexibility and remarkably high biocompatibility. Additionally, PEG can prevent the opsonisation and recognition by the MPS.¹⁰⁻¹² However, the PEGylation of the micelles might prevent the uptake of carriers by the target cells, generate strong immune response

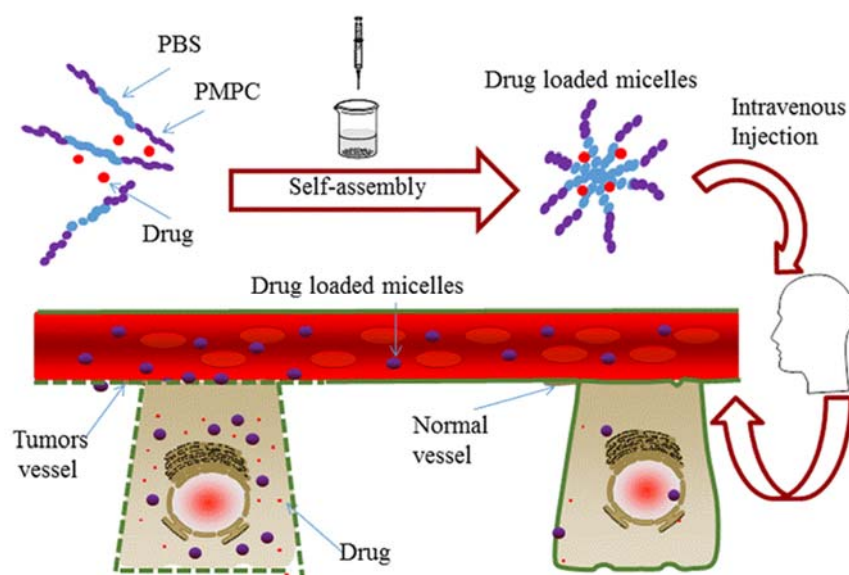
and rapid clearance from the circulation under certain conditions.¹³⁻¹⁵ Therefore, in order to prolong the circulation time of micelles in the bloodstream, developing novel hydrophilic materials with excellent biocompatibility is an urgent issue. Zwitterionic phosphorylcholine (PC) is the hydrophilic head group of cell outer membrane phosphatidylcholine. In previous studies, PC-based polymers are found to exhibit excellent biocompatibility, resistance to protein adsorption and cell adhesion, which endows nanocarriers fabricated by these polymers prolonged circulation time by escaping from the MPS.⁷ Gong et al. developed a series of amphiphilic random copolymers comprised of 2-methacryloyloxyethyl phosphorylcholine (MPC), stearyl methacrylate (SMA) and trimethoxysilylpropyl methacrylate (TSMA), and then prepared stable micelles with PC shells.⁷ *In vivo* studies in New Zealand Rabbits demonstrated that the blood circulation half-life of PMS55 micelles showed an ultra-long circulation time (90.5 h) in the blood. Besides long circulation time, stability and cellular internalization of nanocarriers are also important. Armes et al. proved that a PMPC corona would remarkably enhance the freeze-thaw stability and salt tolerance of polystyrene (PS) nanoparticles.¹⁶ Ishihara et al. reported a multi-functional envelop-type nanodevice coated with copolymer of poly(MPC-*co*-butyl methacrylate) which showed assisted cellular uptake.¹⁷ Ji and co-workers also found that nanoparticles modified with PC showed outstanding stability in plasma and enhanced internalization into cancer cells.^{18,19}

Even so, the greatest limitation of such acrylate polymers was their biodegradability, this may limit their applications as drug carriers. There is no doubt that biodegradable cell membrane mimetic materials have extensive application prospects. Ji et al.¹⁹ and Zhang et al.²⁰ fabricated PC modified aliphatic polyester, polycarbonate and poly(ϵ -caprolactone) micelles, respectively. The results showed that these micelles had great potential for cancer therapy.

Poly(butylene succinate), PBS is a biodegradable and biocompatible linear aliphatic polyester, which synthesized by polycondensation of 1,4-butanediol with succinic acid owns relatively low production cost and properties similar to polyolefin. Furthermore, these two monomers are accessible

from renewable resources, therefore it is considered as a promising polymer to be developed. To our knowledge, there were some works reported about microcapsules, microspheres, scaffolds or films of PBS as drug carriers.²¹⁻²⁴ However, few studies of micelles with PBS as the hydrophobic core have been reported.

In this study, we attempt to develop a micellar drug carrier with hydrophilic PC shells and hydrophobic PBS cores. The schematic illustration of this work was shown in Scheme 1, and the system was designed for the following considerations. First, PBS is both biocompatible and biodegradable. Second, PMPC is highly biocompatible and surface coating MPC-based copolymers are proven to reduce protein absorption and cell adhesion.^{25,26} Furthermore, DOX as a model drug was loaded into the micelles. Cell toxicity, cellular uptake and the phagocytosis by mouse peritoneal macrophage (MPM) of the micelles were also studied.



Scheme 1. Schematic illustration of the preparation of DOX-loaded PMPC-*b*-PBS-*b*-PMPC micelles and the subsequent release of DOX from micelles in tumor and normal vessels.

EXPERIMENTAL

Materials

1,4-Butanediol was dried over anhydrous magnesium sulfate overnight and distilled under reduced pressure before use. Titanium tetraisopropoxide $\text{Ti}(i\text{-OPr})_4$ and 2-bromoisobutyryl bromide both purchased from Aldrich. Doxorubicin hydrochloride ($\text{DOX}\cdot\text{HCl}$) was provided by Hangzhou ICH Biopharm Co., Ltd., Zhejiang, (in China). 6-Coumarin of laser grade was purchased from J & K Reagent Company. L929 cells were obtained from the Fourth Military Medical University. Hela cells were purchased from Sinocelltech Ltd. Bal b/c mice of 6-8 week-old weighing 24 ± 2 g were obtained from Animal Center of Xi'an Jiaotong University. Animals were maintained in accordance with the guidelines of the Shaanxi Provincial Committee for experimental animal management, China, and approved by the Animal Ethics Committee of Northwest University. Fetal bovine serum (FBS), RPMI 1640, DMEM, dimethyl sulfoxide (DMSO) and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) from Sigma were used as received. All the other reagents were of analytical grade and used without further purification.

A cationic amphiphilic copolymer micelles of poly(methacryloxyethyltrimethyl ammonium chloride-*co*-stearyl methacrylate), PDS55 (concentration: 1 mg/mL; size: 117.2 ± 9.3 nm; zeta potential: 14.6 ± 1.3 mV) and PLA nanoparticles (concentration: 1 mg/mL; size: 130.8 ± 5.8 nm; zeta potential: -22.7 ± 1.1 mV) were prepared according to previous reports^{7,27} as reference samples, respectively.

Measurements

^1H NMR spectra were recorded on Varian INOVA 400 MHz spectrometers using CDCl_3 , or the mixture of CDCl_3 and CD_3OD (v/v = 1:1) as solvents. Tetramethylsilane (TMS) was applied as an internal chemical shift reference. FT-IR spectra were recorded on a Bruker Equinox 55 spectrometer via KBr pellet technique under ambient temperature. Molecular weights of the polymer samples were measured on a Perkin-Elmer 200 series GPC instrument (Varian, UK). CHCl_3 was employed as eluent at a flow rate of 1.0 mL/min and a temperature of 40 °C. Polystyrene standards (Showa Denko Ltd, Japan) with narrow molecular weight distributions were employed to make a calibration curve.

Thermal analyses were studied by thermogravimetric analysis (TGA Q500) and differential scanning calorimetry (DSC Q200). Three to five milligrams of samples were scanned from 25 to 600 °C at a heating rate of 10 °C/min under flowing nitrogen. Peak tops (T_d) of the differentiated TGA curve denoted as the $dTGA$ trace were employed as an index to assess thermal degradation behavior and stabilities for the synthesized polymers. For DSC analysis, three to five milligrams of polymer was encapsulated in an aluminum pan and then heated to 180 °C at a heating rate of 10 °C/min. Melting points (T_m) and heat of fusion (ΔH_m) were estimated as the main peak tops and the integrals of the endothermic curve recorded by the DSC run, respectively.

The critical micelle concentration (CMC) of the PMPC-*b*-PBS-*b*-PMPC copolymers in water was determined by a fluorescence probe technique using pyrene as the fluorescent probe. The fluorescence measurement was carried out by a Hitachi F-4500 fluorescence spectrophotometer. The excitation wavelength was 339 nm. The average hydrodynamic diameters and size distributions of the prepared micelle solution were obtained using Malvern Nano-ZS as particle size analyzer (laser, 4 mW, $\lambda = 632$ nm; measurement angle 90 °). The micelle solution was filtrated to remove dust using a microfilter with a nominal pore size of 0.45 μm prior to the measurements and run for three times at 25 °C. TEM (JEM-100CX11, JEOL) was employed to observe the morphology of the PMPC-*b*-PBS-*b*-PMPC micelles. Briefly, a drop of micelle solution (0.2 mg/mL) was placed on a carbon-coated copper grid and further lyophilized for 24 h to characterize.

Synthesis and characterization

Synthesis of the triblock copolymer of PMPC-b-PBS-b-PMPC. The ABA-type triblock copolymer, PMPC-*b*-PBS-*b*-PMPC was synthesized via a three-step synthetic strategy as described below. The synthetic routes of all polymers were shown in Scheme 2.

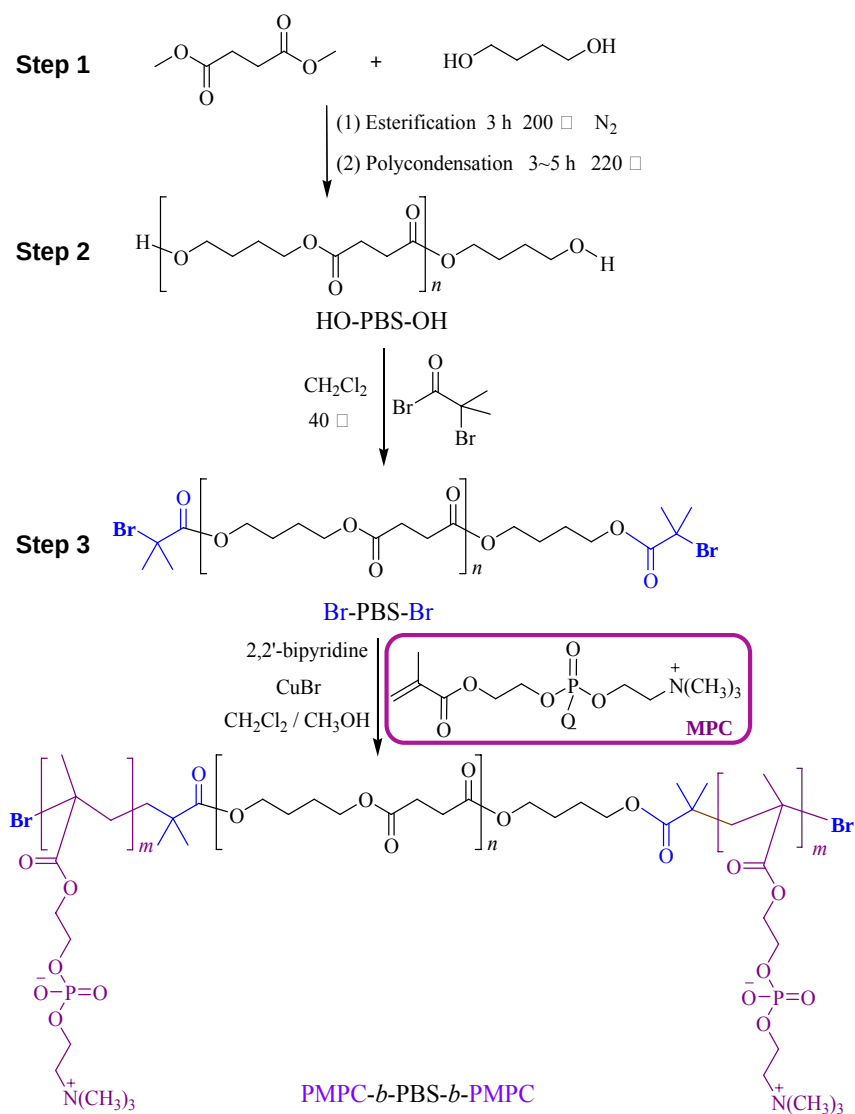
Synthesis of hydroxyl terminated poly(butylene succinate), HO-PBS-OH. Hydroxyl terminated poly(butylene succinate) was synthesized using $\text{Ti}(i\text{-OPr})_4$ as a catalyst referring to our previous report.²⁸ The synthetic route was shown in Scheme 2 (Step 1) and a typical polymerization procedure

was as follows. 2.92 g (20 mmol) dimethyl succinate and 2.16 g (24 mmol) 1,4-butanediol were added into a flame-dried three-necked flask with a gas inlet. Then in the atmosphere of N₂, the flask was transferred to an oil bath under 180 °C for 3 h to remove the esterification byproduct of methanol. After that, 0.02 mmol of Ti(*i*-OPr)₄ were added in as a catalyst and the reaction was further kept for 0.5 h. And then the condensation polymerization was carried out under a gradually reduced pressure at 200 °C for 3~5 h. The crude polymer of HO-PBS-OH was dissolved in chloroform and precipitated in excess amounts of cold methanol three times, and dried at 45 °C in a vacuum oven to constant weight. Yield: 72%. ¹H NMR (400 MHz, CDCl₃): δ 1.71 (-OCH₂CH₂CH₂CH₂O-, 4*n*H, m); 2.62 (-O)CCH₂CH₂C(O)-, 4*n*H, s); 3.67 (-OCH₂CH₂CH₂CH₂OH, 4H, t); 4.15 (-OCH₂CH₂CH₂CH₂O-, 4*n*H, t).

Synthesis of PBS macro-initiator, Br-PBS-Br. Macro-initiator Br-PBS-Br was synthesized by HO-PBS-OH and 2-bromoisobutyryl bromide shown in Scheme 2 (Step 2). In a general procedure: 2.0 g (0.4 mmol) HO-PBS-OH and 0.10 g (1 mmol) anhydrous triethylamine were dissolved in 10 mL anhydrous dichloromethane, and then the solution of 0.4 g (1.6 mmol) 2-bromopropionyl bromide dissolved in 5.0 mL anhydrous dichloromethane was dropped in at room temperature. The reaction was carried out at 40 °C for 24 h. Macro-initiator Br-PBS-Br was precipitated in diethyl ether three times and dried in vacuum oven until constant weight. Yield: 83%. ¹H NMR (400 MHz, CDCl₃) δ 1.71 (-OCH₂CH₂CH₂CH₂O-, 4*n*H, m); 1.95 (-OC(CH₃)₂Br, 12H, s); 2.62 (-O)CCH₂CH₂C(O)-, 4*n*H, s); 4.15 (-OCH₂CH₂CH₂CH₂O-, 4*n*H, t).

Atom transfer radical polymerization of MPC. ATRP was performed to prepare ABA-type triblock copolymer, PMPC-*b*-PBS-*b*-PMPC and the synthetic route was shown in Scheme 2 (Step 3). 0.50 g (0.1 mmol) macro-initiator Br-PBS-Br, a certain amount of MPC and 63 mg (0.4 mmol) 2,2'-bipyridine were dissolved in a mixed solvent of CH₂Cl₂/CH₃OH (v/v = 1:1). Then, the mixture was degassed by three freeze-pump-thaw cycles and purged with Ar gas to eliminate oxygen. After that, 29 mg (0.20 mmol) CuBr, 17 mg (0.11 mmol) 2,2'-bipyridine were added in, respectively. And the

flask was transferred into a 30 °C oil bath for 8 h under an Ar atmosphere. After the reaction, the product was filtered through an alumina column to remove the metal catalyst. The crude copolymer was dissolved in a certain amount of dimethyl formamide (DMF) and dialyzed against 400 mL deionized water at room temperature for 4 d using a dialysis membrane with a molecular weight cut-off of 3500. The external water phase was replaced every 6 h. The copolymer of PMPC-*b*-PBS-*b*-PMPC was obtained by freeze drying. Yield: 69~73%. ¹H NMR (400 MHz, CDCl₃/CD₃OD) 4.38~3.95 (-OCH₂CH₂O-, -OCH₂CH₂N⁺(CH₃)₃-, -OCH₂CH₂CH₂CH₂O-,), 3.75 (-OCH₂CH₂N⁺(CH₃)₃), 3.30 (-N⁺(CH₃)₃, s); 2.62 (-O)CCH₂CH₂C(O)-, s); 1.98 (-OC(CH₃)₂-, s); 1.71 (-OCH₂CH₂CH₂CH₂O-, m); 0.82~1.23 (-CH₂C(CH₃)₂-).



Scheme 2. Synthetic routes of the triblock copolymer of PMPC-*b*-PBS-*b*-PMPC**Fabrication of PMPC-*b*-PBS-*b*-PMPC micelles**

The PMPC-*b*-PBS-*b*-PMPC micelles were obtained by combination of solvent evaporation and dialysis methods. Briefly, 20 mg of the PMPC-*b*-PBS-*b*-PMPC dry powder was dissolved completely in a mixed solution of CHCl₃/CH₃OH (*v/v* = 1:1) at a concentration of 8 mg/mL in a glass vial at room temperature. Then, the solution was added dropwise to 25 mL deionized water under vigorous stirring. The micelles were fabricated through the evaporation overnight and dialyzed for 3 days to completely remove CHCl₃ and CH₃OH. After that, the resultant solution was centrifuged (AnkeGL-20G-II) for 10 min at the speed of 7980 rpm and the supernatant was passed through a 0.45 µm syringe filter to obtain the micelle solution of PMPC-*b*-PBS-*b*-PMPC.

Stability of PMPC-*b*-PBS-*b*-PMPC micelles

The stability of the PMPC-*b*-PBS-*b*-PMPC micelles was studied by preparing micelles solutions (0.8 mg/mL) at phosphate buffer pH 7.4 for a week at 4 °C storage in a refrigerator. Then, the changes of sizes and distributions were measured by DLS.

DOX loading and *in vitro* DOX release study

Dox-loaded PMPC-*b*-PBS-*b*-PMPC micelles were prepared using the same procedure as described above, except 5 mg DOX·HCl and 2 equiv. of triethylamine were dissolved in the mixed solution of CHCl₃/CH₃OH (*v/v*=1:1). The theoretical drug loading content was set at 20 wt.%. After the solvent was evaporated completely, the residual solution was centrifuged for 10 min at the speed of 7980 rpm followed by filtration using 0.45 µm syringe filter to remove the undissolved DOX and aggregated micelles. Finally, the micelle solution was diluted with distilled water to obtain desired concentrations (0.8 mg/mL). For the determination of drug loading content (LC) and encapsulation efficiency (EE), the DOX loaded micelles solution was freeze-dried, dissolved in the mixed solution of CHCl₃/CH₃OH, and analyzed by UV-Vis spectroscopy, wherein a calibration curve was obtained

with DOX in the mixed solution of $\text{CHCl}_3/\text{CH}_3\text{OH}$ with different concentrations. The calculation formulas of LC and EE were as previous report.²⁰

The release profile of DOX from PMPC-*b*-PBS-*b*-PMPC micelles was studied using a dialysis tube (MWCO 3500) in 25 mL phosphate buffer pH 7.4 or 5.5 at 37 °C. At predetermined time intervals, 5.0 mL release media were taken out and replaced by an equal volume of fresh media. The amount of DOX released was determined by UV spectrophotometer based on the absorbance intensity at 480 nm against a standard calibration curve. And the accumulative release (%) was calculated as previous report.²⁰

***In vitro* cytotoxicity assay**

The *in vitro* cytotoxicity of the PMPC-*b*-PBS-*b*-PMPC micelles to L929 (normal cell) and Hela (cancer cell) cells was studied by MTT assay.^{7,20} The cells were seeded at a density of 2×10^3 cells per well in 100 μL of RPMI 1640 medium containing 10% FBS in 96-well plates and grown for 24 h (37 °C, 5% CO_2). The culture medium was replaced by 100 μL micelle suspensions with different concentrations of 0.05, 0.2 and 0.8 mg/mL, and then the cells were incubated for another 24 h. After that, the micelle solutions were replaced by 100 μL fresh culture medium, and 20 μL MTT solutions (5 mg/mL) was added. After 4 h incubation, 100 μL /well dimethyl sulfoxide (DMSO) was added to dissolve the formazan crystals for 10 min. The optical density (OD) in each well was detected and the cell viability was calculated by the following equation:

$$\text{Cell viability (\%)} = (\text{OD}_{\text{sample}}/\text{OD}_{\text{control}}) \times 100\% \quad (1)$$

where the $\text{OD}_{\text{sample}}$ and $\text{OD}_{\text{control}}$ was detected by the Sunrise automatic reader (TitertekPlusMS212, ICN, Eschwege, Germany) at 490 nm.

In addition, *in vitro* cytotoxicity of the DOX loaded PMPC₆₄-*b*-PBS-*b*-PMPC₆₄ micelles to Hela cells were also studied by the same method using free DOX with different concentrations as a control.

Cellular uptake analysis

By flow cytometry, the cell internalization of free DOX and DOX-loaded PMPC₆₄-*b*-PBS-*b*-PMPC₆₄ micelles were evaluated as previous report.²⁰ In brief, HeLa cells were seeded in 24-well plates at 2×10^5 cells per well and incubated for 18 h at 37 °C. Then, the medium was replaced with free DOX or DOX loaded micelles in fresh culture media at an equivalent DOX concentration of 8 µg/mL and incubated at 37 °C. After being incubated for 2 h, the culture medium was removed and the cells were washed three times with phosphate buffered solution and trypsinized for FACS analyses. Cells with PBS treatment were used as the control. The laser source wavelength of FACS (BD Biosciences) is 488 nm ($\lambda_{\text{ex, DOX}} = 480$ nm), and a total 10 000 cells were collected and analysed in each experiment.

***In vitro* phagocytic uptake test**

The mouse peritoneal macrophage (MPM) was employed to evaluate the phagocytic uptake of 6-coumarin-loaded PMPC-*b*-PBS-*b*-PMPC polymeric micelles in vitro. The preparation of 6-coumarin-loaded micelles, was exactly the same as that of DOX-loaded micelles, except that DOX base (5 mg/ml) chloroform solution was replaced by 10 µL 6-coumarin (0.45 mg/mL) chloroform solution. And the concentration of 6-coumarin-loaded micelles was 0.2 mg/mL ($m_{\text{polymer}}:m_{\text{6-coumarin}} = 1000:1$). The elicited macrophages were harvested from the bal b/c mice after intraperitoneal injection of 1 mL/day of sterile starch broth for 3 days to produce a large number of macrophages.^{20,29} Resulted cells were isolated by centrifugation at 1000 rpm for 5 min and then re-suspended in RPMI 1640 supplemented with 10% heat-inactivated FBS. Thereafter, the cells were seeded in 24-well culture plates at a density of 1.0×10^6 cells/mL and incubated in a humidified atmosphere containing 5% CO₂ at 37 °C to allow cell adherence.

After 24 h incubation, the culture medium was replaced by 1.0 mL 0.2 mg/mL suspensions of 6-coumarin loaded micelles in culture medium and incubated with the cells at 37 °C for 2 h. After that, the micelle suspension in the testing wells was sucked out and the wells were washed with 0.5 mL phosphate buffer solution for three times to ensure the removal of micelles adhered to the cell

surface. The polymeric micelles phagocytized by MPM were visualized using an inverted fluorescence microscope (Olympus IX71).

For quantification analysis,^{20,29} the culture medium containing 6-coumarin micelles after the MPM uptakes was centrifuged at 1500 rpm for 10 min, and the supernatant was measured with a fluorescence spectrophotometer to read the intensity of the fluorescence emission peak at 500 nm (λ_{ex} = 430 nm). The macrophage uptake ratio was calculated by the following equation:

$$\text{Uptake ratio} = (I_0 - I) / I_0 \times 100\% \quad (2)$$

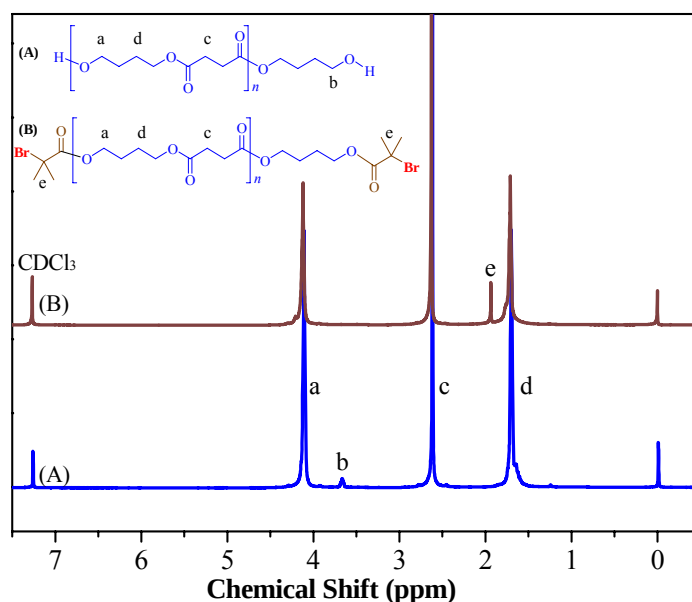
where I_0 and I are the fluorescence intensity of the culture medium before and after phagocytosis, respectively.

RESULTS AND DISCUSSIONS

Synthesis and characterization of the triblock copolymer PMPC-*b*-PBS-*b*-PMPC

The synthetic routes of triblock copolymer of PMPC-*b*-PBS-*b*-PMPC are illustrated in Scheme 2. In the first step, HO-PBS-OH polymer was prepared by the condensation polymerization using Ti(*i*-OPr)₄ as the catalyst. The molar ratio of dimethyl succinate and 1,4-butanediol was 1/1.2 to obtain PBS with terminal hydroxyl groups, HO-PBS-OH.^{28,30} ¹H NMR signals (Fig. 1A) of HO-PBS-OH at δ 4.15, 3.67, 2.62 and 1.71 ppm were respectively assigned to the protons of -OCH₂CH₂CH₂CH₂O-, -CH₂OH, -(O)CCH₂CH₂C(O)- and -OCH₂CH₂CH₂CH₂O- as reported.²⁸ Moreover, the degree of polymerization (n) of HO-PBS-OH was calculated from this ¹H NMR spectrum according to the ratio of the signals at δ 4.15 and 3.67 ppm, which was found to be 28. The subsequent end-functionalization of polymer HO-PBS-OH with 2-bromoisobutryl bromide yielded Br-PBS-Br in the presence of trimethylamine as the acid acceptor. Fig 1B shows the ¹H NMR spectrum of the polymer Br-PBS-Br. As shown that there was only a small difference between the ¹H NMR spectra of HO-PBS-OH and Br-PBS-Br. The signal of -CH₂OH at δ 3.67 ppm in Fig. 1A could not be observed, suggesting that all the hydroxyl groups of HO-PBS-OH have been reacted. Meanwhile, a new single resonance peak was observed at δ 1.95 ppm, which was assigned to the methyl protons

(signal e) of 2-bromoisobutyryl groups.^{31,32} Finally, the copolymer of PMPC-*b*-PBS-*b*-PMPC was synthesized by ATRP using Br-PBS-Br as a macro-initiator. The polymerization of the methacrylate monomer MPC was carried out in a mixed solvent of CH₂Cl₂/CH₃OH (*v/v* = 1:1) using CuBr as a catalyst and 2,2'-bipyridine as a ligand. The copolymer was purified by filtration through the alumina column and dialyzed against deionized water. The ¹H NMR spectrum of the PMPC-*b*-PBS-*b*-PMPC triblock copolymers was acquired in the mixed solution of CDCl₃/CD₃OD (*v/v* = 1:1), which contained signals from protons of the PBS (δ 4.15, 2.62, 1.71 ppm) and PMPC (δ 4.38~3.95, 3.75, 3.30, 1.98, 0.82~1.23 ppm) moieties (Fig. 1C). The molecular weight of the PMPC-*b*-PBS-*b*-PMPC copolymer was determined by comparing the integrals at δ 3.75 (methylene protons of PMPC moiety) to that at 2.62 (methylene protons of PBS moiety). To establish a structure-function relationship, three different proportion copolymers were synthesized and characterized. Three block copolymers with different length of PMPC were prepared and would be named as PMPC₁₁-*b*-PBS-*b*-PMPC₁₁, PMPC₂₅-*b*-PBS-*b*-PMPC₂₅ and PMPC₆₄-*b*-PBS-*b*-PMPC₆₄, with the subscript indicating the number-average degree of polymerization of the block.



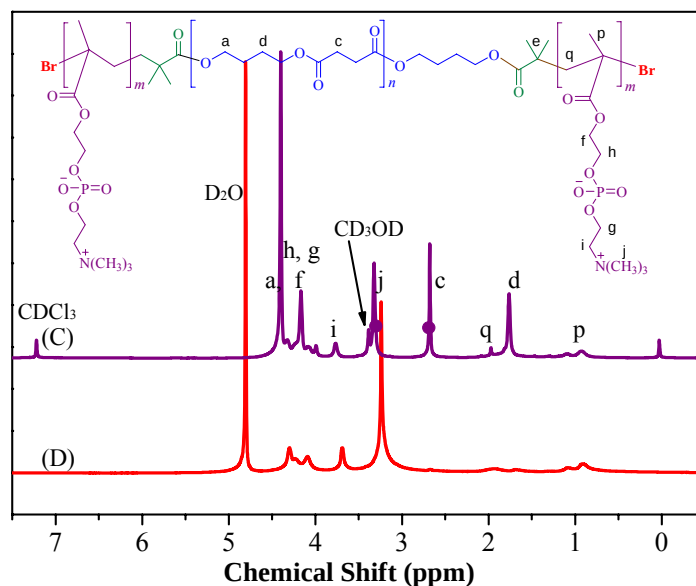


Figure 1 ^1H NMR spectra recorded at 25 °C for (A) HO-PBS-OH in CDCl_3 ; (B) Br-PBS-Br in CDCl_3 ; (C) $\text{PMPC}_{11}\text{-}b\text{-PBS-}b\text{-PMPC}_{11}$ in a 1:1 $\text{CDCl}_3/\text{CD}_3\text{OD}$ mixture (which is a good solvent for both blocks); (D) $\text{PMPC}_{11}\text{-}b\text{-PBS-}b\text{-PMPC}_{11}$ polymeric micelles in D_2O .

The triblock copolymers were further characterized by FT-IR and GPC. Compared to the FT-IR spectra (Fig. S1) of HO-PBS-OH and Br-PBS-Br, the newly emerging absorbance peaks at 1245, 1080 and 972 cm^{-1} in the FT-IR spectrum of $\text{PMPC}_{11}\text{-}b\text{-PBS-}b\text{-PMPC}_{11}$ were assigned to the stretching vibration of P=O , P-O and $-\text{N}^+(\text{CH}_3)_3$ in the PMPC chain, respectively.³³ The results of polymerization and molecular weight analysis were summarized in Table 1. GPC in CHCl_3 revealed that copolymer $\text{PMPC}_{11}\text{-}b\text{-PBS-}b\text{-PMPC}_{11}$ had a relatively low polydispersity index of 1.11 and an M_n of 10.8 kg/mol, close to that determined by ^1H NMR (11.3 kg/mol), confirming the controlled synthesis of this triblock copolymer. Single solvent such as THF, DMSO and CHCl_3 could not dissolve copolymers of $\text{PMPC}_{25}\text{-}b\text{-PBS-}b\text{-PMPC}_{25}$ and $\text{PMPC}_{64}\text{-}b\text{-PBS-}b\text{-PMPC}_{64}$ as a unimer state, thus GPC data of these two copolymers could not be detected.

Thermal characterization of HO-PBS-OH and $\text{PMPC-}b\text{-PBS-}b\text{-PMPC}$

Thermal degradation behavior is of great importance for application of biodegradable polymers. Here, peak temperatures of the corresponding differentiated TGA traces ($dTGA$, T_d) were applied to

make a qualitative assay of thermal stabilities of the polymers of HO-PBS-OH and PMPC-*b*-PBS-*b*-PMPC. And the results are summarized in Table 1 and the profiles are shown in Fig. S2. It can be seen that the linear HO-PBS-OH showed a high T_d of 385 °C as previously reported.²⁸ However, there are two or three T_d peaks in the $dTGA$ traces of the block copolymers of PMPC-*b*-PBS-*b*-PMPC which obviously shifted toward the lower-temperature side, demonstrating a lower thermal stability for these copolymers. Meanwhile, the weight loss at 70~80 °C in the $dTGA$ traces of the block copolymers of PMPC-*b*-PBS-*b*-PMPC might from the loss of absorbed water due to the strong hydrophilicity of PMPC segment.

Crystallinity of the amphiphilic copolymers as an ideal drug carrier has a great influence on drug incorporation, micelle stability, and drug release. Generally, an amorphous structure is favourable for drug loading, while a crystalline structure provides good kinetic stability and sustained drug release of micelles.³⁴⁻³⁶ Fig. 2 depicted DSC curves for the copolymers of PMPC-*b*-PBS-*b*-PMPC with different PMPC lengths and that of HO-PBS-OH was used for comparison. Polymer HO-PBS-OH exhibited a melting point at around 113 °C as ever reported²⁸ and melting enthalpy $\Delta H_m = 55.9$ J/g. Moreover, the melting temperature for copolymers of PMPC₁₁-*b*-PBS-*b*-PMPC₁₁ and PMPC₂₅-*b*-PBS-*b*-PMPC₂₅ was detected at around 112 °C, corresponding to the melting temperature of PBS segments in the copolymers of PMPC-*b*-PBS-*b*-PMPC. This indicates that phase separation takes place in these two block copolymers.³⁷ However, the melting enthalpy ΔH_m of these block copolymers decreased significantly. Even the T_m and ΔH_m of copolymers of PMPC₆₄-*b*-PBS-*b*-PMPC₆₄ were not observed. As a result, crystallization of the PBS chains is affected by the introduction of PMPC chains. The lower crystallinity of the PBS blocks within the copolymers of PMPC-*b*-PBS-*b*-PMPC may be advantageous in terms of the drug loading of PMPC-*b*-PBS-*b*-PMPC micelles.

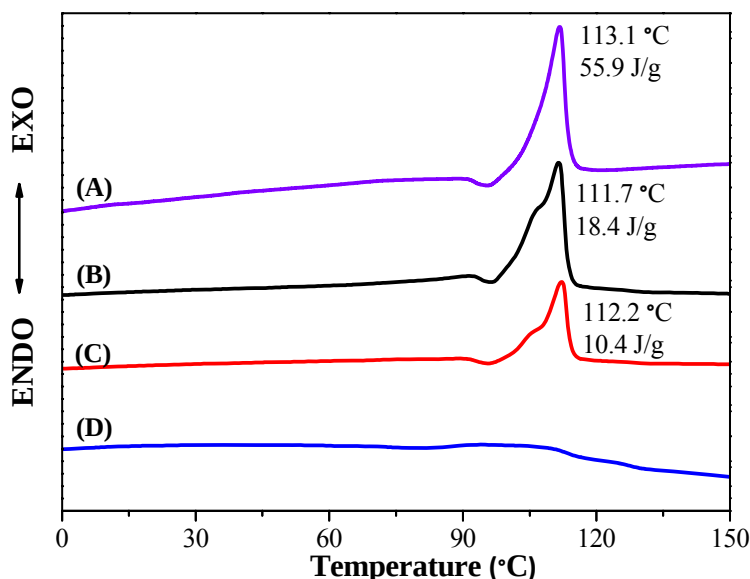


Figure 2 DSC thermograms for the polymers of HO-PBS-OH (A), PMPC₁₁-*b*-PBS-*b*-PMPC₁₁ (B), PMPC₂₅-*b*-PBS-*b*-PMPC₂₅ (C) and PMPC₆₄-*b*-PBS-*b*-PMPC₆₄ (D).

Formation of PMPC-*b*-PBS-*b*-PMPC micelles

It is well known that amphiphilic copolymer can form core-shell type micelles by self-assembly when exposed to a selective solvent. In this study, the amphiphilic PMPC-*b*-PBS-*b*-PMPC triblock copolymer micelles were obtained through combination of solvent evaporation and dialysis treatments. The core-shell structure of the micelles is confirmed based on the evidence from ¹H NMR measurement. ¹H NMR spectrum of PMPC-*b*-PBS-*b*-PMPC in CDCl₃/CD₃OD showed characteristic signals of protons from the PMPC and PBS blocks (Fig. 1C). However, that of the freeze-dried powder of PMPC-*b*-PBS-*b*-PMPC micelles in D₂O showed the typical signals only from the PMPC block (Fig. 1D), suggesting that the PMPC block is the hydrated shell and the PBS block forms the dehydrated micellar core.

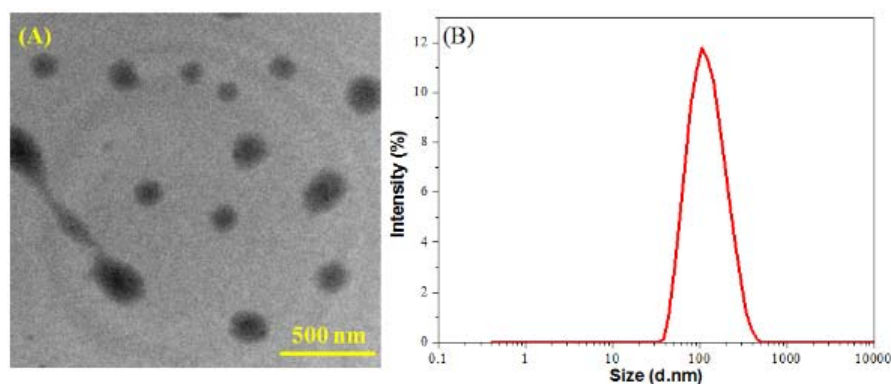


Figure 3 Size distribution profiles of PMPC₁₁-*b*-PBS-*b*-PMPC₁₁ micelles in distilled water determined by TEM (A), and DLS measurement (B).

Moreover, the size and shape of the PMPC-*b*-PBS-*b*-PMPC micelles were evaluated by DLS and TEM measurements, respectively. As demonstrated in Table 2 and Fig. 3A, the results of DLS showed that PMPC-*b*-PBS-*b*-PMPC formed micelles with sizes that ranging from 108 to 170 nm and PDI of 0.18~0.20. And the average size of the micelles increased with the length of the hydrophilic PMPC block. The longer hydrophilic PMPC block will lead to thicker hydration shell when the length of the hydrophobic PBS block was constant. TEM micrograph revealed that these micelles had a spherical morphology (Fig. 3B). However, some aggregates are detected by TEM. We speculated that aggregates formed during the freeze-drying process. The CMC of the PMPC-*b*-PBS-*b*-PMPC copolymers were measured by a fluorescence spectrophotometer using pyrene (the pyrene concentration in the final solutions: 3.5×10^{-7} mol/L; the concentrations of PMPC-*b*-PBS-*b*-PMPC micelle solutions ranging from 1.3×10^{-6} to 5.0×10^{-1} mg/mL) as a hydrophobic probe, which ranged from 1.2 to 3.6 mg/L. Fig. S3 showed the fluorescence intensity ratio I_{384}/I_{375} of as a function of concentration to evaluate the CMC of micelles prepared from copolymer PMPC₆₄-*b*-PBS-*b*-PMPC₆₄. Compared with the CMC of lower molecular weight surfactants such as sodium lauryl sulfate (SLS, CMC=2.5 g/L), the CMC of the PMPC-*b*-PBS-*b*-PMPC copolymers was much lower, suggesting that these PMPC-*b*-PBS-*b*-PMPC polymeric micelles might be stable and could serve as long circulation carriers for drug delivery.

Stability of PMPC-*b*-PBS-*b*-PMPC micelles

The stability of the PMPC-*b*-PBS-*b*-PMPC micelles was studied by determining the changes of sizes and distributions of micelles solutions stored for one week at phosphate buffer pH 7.4 at 4 °C (storage in a refrigerator). As shown in Table S1 and Fig. S4, the sizes and distributions of micelles solutions of all three copolymers did not change significantly before and after storage, indicating that the micelles were stability and could serve as long circulation carriers for drug delivery, which might be attributed to the zwitterionic PC shells of the micelles.

Loading and *in vitro* DOX release from PMPC-*b*-PBS-*b*-PMPC micelles

DOX is a potential anticancer drug widely used for treatment various types of solid malignant tumors.³⁸ It is often used as a hydrophobic drug model to demonstrate utility of various drug delivery systems, including polymeric particles and other polymer-drug conjugates.^{9,10,38}

Here, DOX was loaded into the PMPC-*b*-PBS-*b*-PMPC micelles and the theoretical drug loading content was set at 20 wt.%. The results (Table 2 and Fig. S4) showed that after loading of DOX, PMPC-*b*-PBS-*b*-PMPC micelles had larger sizes, but still smaller than 200 nm which rendered them possible to escape from the reticuloendothelial system (RES) and to accumulate at the tumor sites by the enhanced permeability and retention (EPR) effect.¹ And the drug loading content (LC) and encapsulation efficiency (EE) of DOX loaded PMPC-*b*-PBS-*b*-PMPC micelles calculated as described previously^{2,17} were about 12~13% and 41% which were independent of their composition.

The release behavior of DOX from DOX-loaded PMPC-*b*-PBS-*b*-PMPC micelles were investigated using a dialysis tube (MWCO 3500) at phosphate buffer pH 7.4 and 5.5 media at 37 °C, as shown in Fig. 4. The results showed that the DOX release rate from micelles accelerated when pH decreasing from 7.4 to 5.5. For example, the total amount of DOX released from PMPC₆₄-*b*-PBS-*b*-PMPC₆₄ micelles

Table 1 ATRP results and characterization of copolymer PMPC-*b*-PBS-*b*-PMPC

Polymer ^a	PBS ^b /MPC feeding molar ratio	BS/MPC unit molar ratio in copolymer ^c	Yield (%)	M_n^e ($\times 10^{-4}$)	M_n^f ($\times 10^{-4}$)	PDI ^f	T_d^h (°C)
PMPC ₁₁ - <i>b</i> -PBS- <i>b</i> -PMPC ₁₁	1:25	28 ^d :22	70	1.13	1.08	1.11	268 345
PMPC ₂₅ - <i>b</i> -PBS- <i>b</i> -PMPC ₂₅	1:50	28:50	73	1.96	- ^g	-	268 337
PMPC ₆₄ - <i>b</i> -PBS- <i>b</i> -PMPC ₆₄	1:100	28:128	69	4.26	-	-	276 337 378

^a All polymers were synthesized by ATRP. ^b PBS were synthesized by the condensation polymerization under 200~220 °C with titanium tetraisopropoxide as the catalyst, and the degree of polymerization (*n*) of HO-PBS-OH was calculated from ¹H NMR spectrum according to the ratio of the integrals at δ 4.15 and 3.67 ppm. ^c The BS/MPC unit molar ratios in copolymer were estimated by ¹H NMR by comparing the integrals at δ 3.75 (methylene protons of PMPC moiety) to that at 2.62 (methylene protons of PBS moiety). ^d Indicates the degree of polymerization of HO-PBS-OH. ^e Determined by ¹H NMR in CDCl₃/CD₃OD. ^f Determined by GPC using chloroform as eluent based on PS standards. ^g “—” means that the item could not be measured or detected. ^h The thermal degradation temperature (T_d , °C) were measured by TG with a scanning rate of 10 °C/min.

Table 2 The properties of PMPC-*b*-PBS-*b*-PMPC micelles with and without DOX. Each value was expressed as mean $\pm$ SD from three independent measurements.

Polymer	DOX-free micelles		DOX-loaded micelles		LC ^b	EE ^b
	Size (nm) ^a	PDI ^a	Size (nm) ^a	PDI ^a	(%)	(%)
PMPC ₁₁ - <i>b</i> -PBS- <i>b</i> -PMPC ₁₁	108.4 $\pm$ 2.6	0.181	149.8 $\pm$ 0.6	0.215	13.3	41.5
PMPC ₂₅ - <i>b</i> -PBS- <i>b</i> -PMPC ₂₅	124.3 $\pm$ 2.3	0.190	165.6 $\pm$ 2.8	0.286	12.6	41.1
PMPC ₆₄ - <i>b</i> -PBS- <i>b</i> -PMPC ₆₄	157.6 $\pm$ 2.9	0.200	193.5 $\pm$ 1.7	0.256	12.3	41.0

^aThe average size and polydispersity index (PDI) of micelles determined by dynamic light scattering (DLS) measurements. ^bThe drug loading content (LC) and drug encapsulation efficiency (EE) determined by UV-Vis analyses.

at phosphate buffer pH 7.4 only achieved 21.2% after 10 h. In contrast, the DOX release rate was much faster at a lower pH (pH 5.5), with about 34.6% of DOX being released in 10 h. This suggested that DOX was released in a slow and sustained manner under physiological conditions, which could help to minimize the leakage of DOX in the blood, substantially reducing the unwanted side effects during blood circulation. The different release rate for DOX loaded PMPC-*b*-PBS-*b*-PMPC micelles at different pH media could be considered as the solubility of DOX, the chain length of PMPC and the degradation rate of PBS. Lower pH significantly increased the solubility of DOX, moreover longer PMPC chain length (more hydrophilic) and faster degradation of PBS chain in lower pH media improved the water infiltration of micelles, and ultimately resulted in faster release.

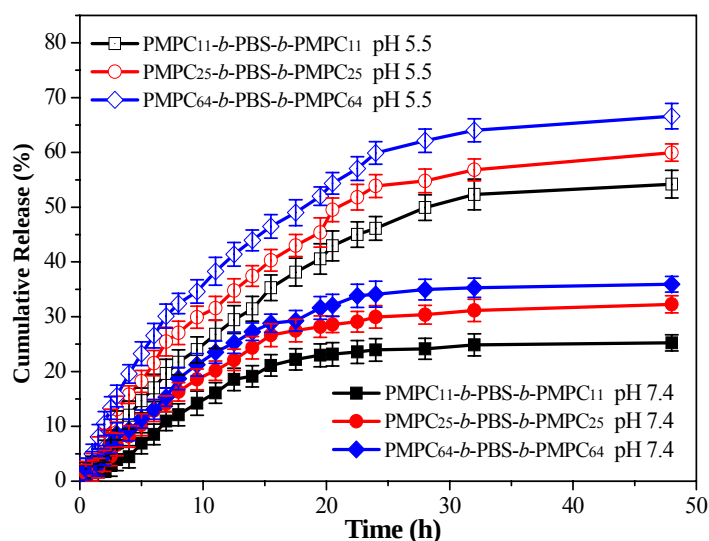


Figure 4 *In vitro* release profiles of DOX loaded PMPC-*b*-PBS-*b*-PMPC micelles in phosphate buffer pH 7.4 and pH 5.5 at 37 °C, respectively.

***In vitro* cell viability study**

In order to investigate the biocompatibility of PMPC-*b*-PBS-*b*-PMPC micelles, *in vitro* cell viabilities of micelles against normal cells (L929) and cancer cells (Hela) were studied using MTT assay. Phenol was used as a control sample. The effects of micelle concentrations on the cell viability are shown in Fig. 5.

The results showed that after 24 h incubation, the phenol showed significant toxicity to L929 and Hela cells at the concentration of 0.2 mg/mL, but the cells viabilities of all the PMPC-*b*-PBS-*b*-PMPC micelles to L929 cells were higher than 90% and decreased slightly with the increasing concentrations of polymeric micelles. This result confirmed the excellent biocompatibility of PMPC-*b*-PBS-*b*-PMPC micelles to L929 cells, providing strong support for potential use *in vivo*. By contrast, Hela cells showed lower cell viability than with healthy cells (L929) in the presence of the PMPC-*b*-PBS-*b*-PMPC micelles.

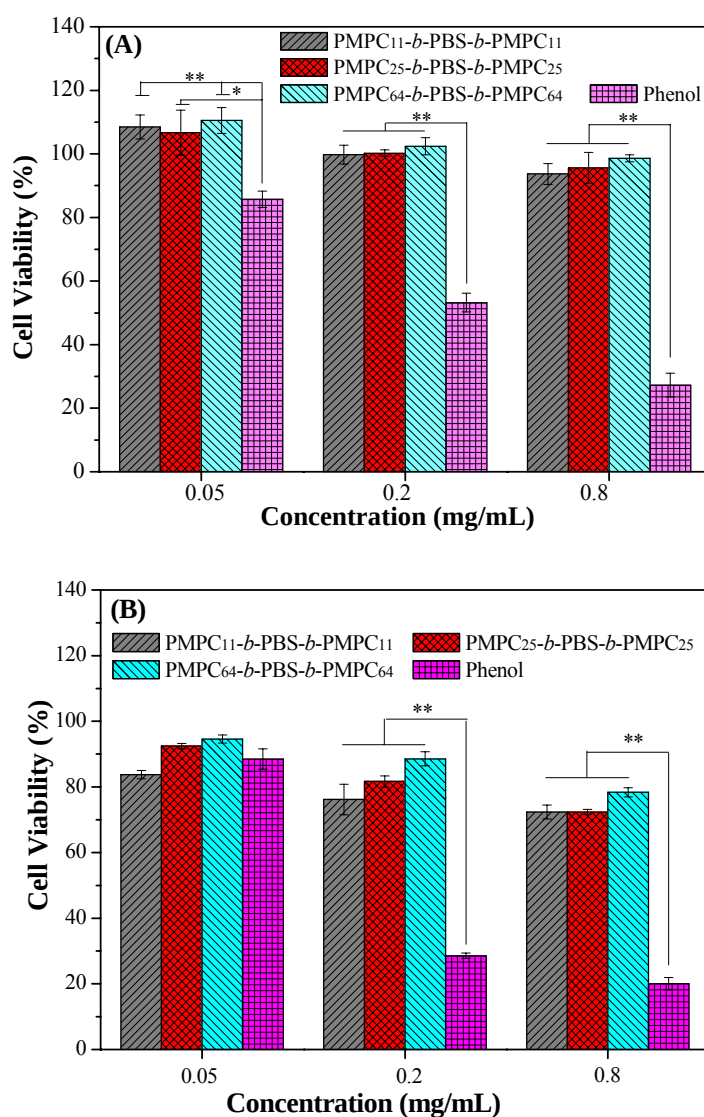


Figure 5 *In vitro* cell viabilities of PMPC-*b*-PBS-*b*-PMPC micelles on L929 (A) and Hela (B) cells were determined by MTT assay after 24 h incubation at 37 °C. Cell viability (%) was determined

based on the ratio of optical density of treated cells to that of the untreated cells. Data were expressed as mean $\pm$ SD, $n=4$. * $p \leq 0.05$ and ** $p \leq 0.01$ versus that of the phenol group.

As shown in Fig. 5B, blank PMPC₆₄-*b*-PBS-*b*-PMPC₆₄ micelles showed 95~78% cell viability to Hela cells after 24 h treatment, indicating relatively good compatibility to Hela cells. We next investigated *in vitro* cytotoxicity of DOX-loaded PMPC₆₄-*b*-PBS-*b*-PMPC₆₄ micelles compared with that of free DOX in Hela cells using the MTT assay. The results (see in Fig. 6) showed that DOX-loaded PMPC₆₄-*b*-PBS-*b*-PMPC₆₄ micelles displayed obvious cytotoxicity (75~36% cell viability) in Hela cells, and free DOX (50~35% cell viability) showed higher cytotoxicity at all concentrations. This can be attributed to the rapid diffusion in cells of DOX due to its smaller size resulting in higher accumulation and thus higher cytotoxicity. However, slowly internalized in the cells and time-consuming release of DOX-loaded polymeric micelles are responsible for relatively higher cell viability. And similar observations have been reported by many researchers.³⁹⁻⁴¹

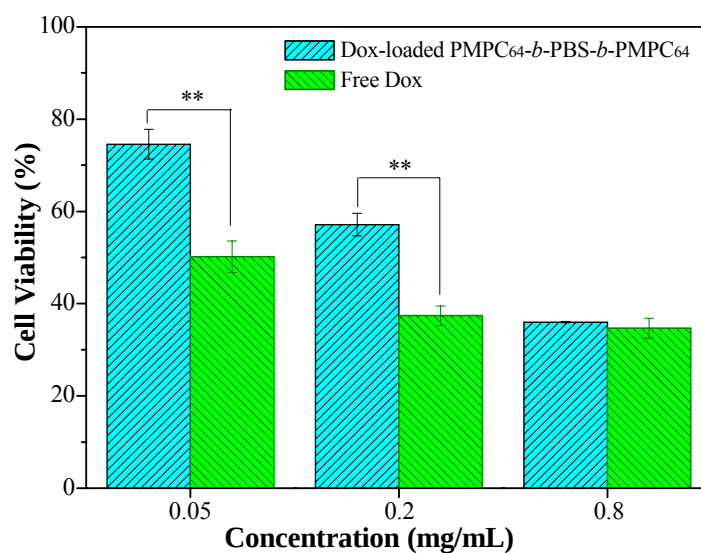
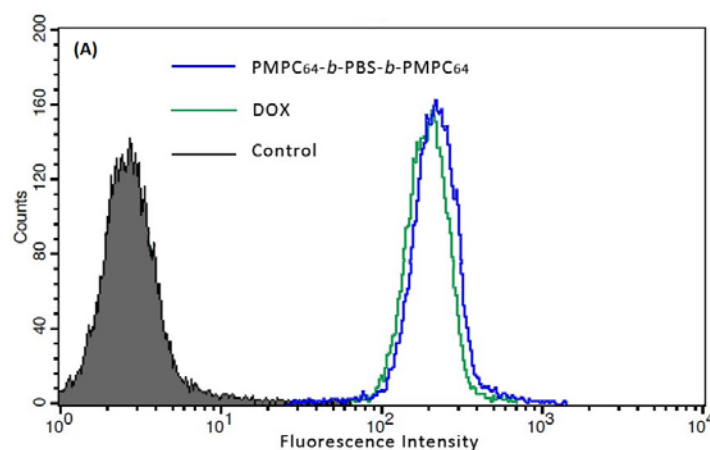


Figure 6 *In vitro* viabilities of free Dox and Dox-loaded PMPC₆₄-*b*-PBS-*b*-PMPC₆₄ micelles on Hela cells determined by the MTT assay after 24 h incubation at 37 °C. All the micelles were loaded roughly with 12~13% of DOX, and the same amount of free DOX were added as in the control

samples. Cell viability (%) was determined based on the ratio of optical density of treated cells to that of the untreated cells. Data were expressed as mean $\pm$ SD, $n=4$.

Cellular uptake

Furthermore, the *in vitro* cellular uptakes of free DOX and DOX-loaded PMPC₆₄-*b*-PBS-*b*-PMPC₆₄ micelles in HeLa cells were studied using flow cytometry and inverted fluorescence microscopy. Fig. 7 showed the flow cytometry histograms and the results of mean fluorescence intensities. Compared to the blank cells, the histograms of HeLa cells incubated with free DOX and DOX-loaded PMPC₆₄-*b*-PBS-*b*-PMPC₆₄ micelles were shifted significantly to the direction of high fluorescence intensity (Fig. 7A), and the mean fluorescence signals for the free DOX and DOX-loaded PMPC₆₄-*b*-PBS-*b*-PMPC₆₄ micelles were stronger than that of blank cells (Fig. 7B), which demonstrating that the DOX-loaded micelles were uptake by HeLa cells successfully. Moreover, DOX-loaded PMPC₆₄-*b*-PBS-*b*-PMPC₆₄ micelles exhibited similar or slightly greater intracellular fluorescence relative to free DOX, this result maybe come from different internalized mechanisms.⁴² DOX molecules entered the cells through passive diffusion and further diffused into the nuclei, while the polymeric micelles were internalized by endocytosis. The results from inverted fluorescence microscopy (Fig. S5) were consistent with those of flow cytometry uptake assay, confirming that the DOX-loaded PMPC₆₄-*b*-PBS-*b*-PMPC₆₄ micelles were internalized in HeLa cells.



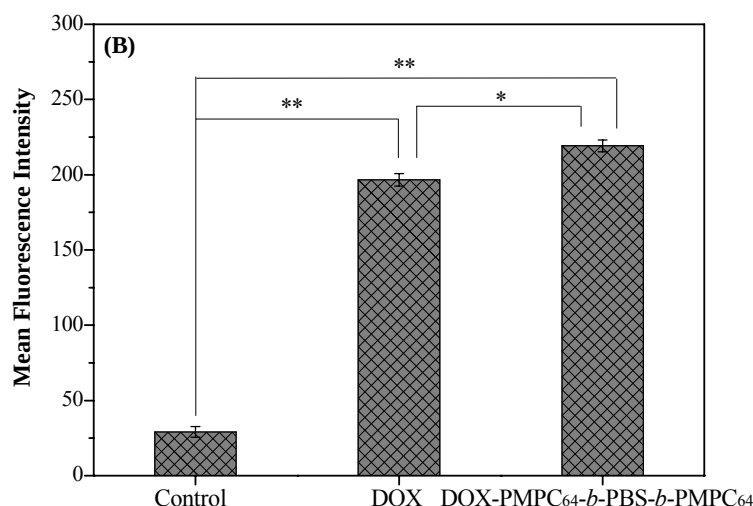


Figure 7 The flow cytometry histograms (A), and the mean fluorescence intensities (B) of cellular uptake of HeLa cells after incubation with free DOX or DOX-loaded PMPC-*b*-PBS-*b*-PMPC micelles for 2 h at a DOX concentration of $8 \mu\text{g mL}^{-1}$. Data represent mean $\pm$ SD ($n = 3$). * $p \leq 0.05$ and ** $p \leq 0.01$ versus that of the control group.

***In vitro* phagocytic uptake test**

It is crucial to find out whether these polymer micelles can be taken up by macrophages. In this paper, the PMPC-*b*-PBS-*b*-PMPC micelles clearance by MPM was evaluated using coumarin-6 as a fluorescence probe. Moreover, positively charged PDS55 micelles⁷ and negatively charged PLA nanoparticles²⁷ were used as the control samples for comparison. The phagocytic uptakes of the coumarin-6 loaded PMPC-*b*-PBS-*b*-PMPC micelles by MPM were visualized using an inverted fluorescence microscope. It should be stressed that the polymer micelles or nanoparticles were loaded with almost the same concentration of 6-coumarin probes and the images were taken under exactly the same conditions. As shown in Fig. 8, the PDS55 and PLA nanoparticles as incubated control groups exhibited brighter green fluorescence compared to the coumarin-6-loaded PMPC-*b*-PBS-*b*-PMPC micelles groups. Moreover, the fluorescence intensity was decreased with increasing of PMPC segment in different coumarin-6-loaded PMPC-*b*-PBS-*b*-PMPC micelles. These results can be explained that the uptake of polymeric micelles by the reticuloendothelial system *in vivo* was

related to surface properties. The stronger the surface hydrophobicity, the more easily it would be recognized by the RES.^{7,43} So, the cellular uptake efficiency of hydrophobic PLA and PDS55 particles was significantly higher. As the PMPC-*b*-PBS-*b*-PMPC copolymer, contains more molar units of PMPC chains with an increasing block length of PMPC segment, the PMPC-*b*-PBS-*b*-PMPC micelles should exhibit more hydrophilicity. Therefore, the cellular uptake efficiency of PMPC-*b*-PBS-*b*-PMPC micelles was decreased with increasing block length of PMPC segment.

Meanwhile, the phagocytic uptake percentage was further quantified by measuring the fluorescence intensities of the micelles containing cell culture solutions before and after the cellular uptake. The results shown in Fig. 9 exhibited that compared to the PDS55 and PLA nanoparticles, the uptake percentage of the coumarin-6-loaded PMPC-*b*-PBS-*b*-PMPC micelles groups decreased significantly. In particular for the PMPC₆₄-*b*-PBS-*b*-PMPC₆₄ micelles, near 3.9-fold and 5.5-fold decrease of the macrophage cellular uptake compared to PDS55 and PLA nanoparticles were observed. We can conclude that PMPC-*b*-PBS-*b*-PMPC micelles show a good performance in avoiding the phagocytosis of phagocyte. As the result, these biodegradable micelles fabricated by copolymers of PMPC-*b*-PBS-*b*-PMPC may have potential advantages for developing long circulation and controlled release nano-carriers for more effective drug delivery systems.

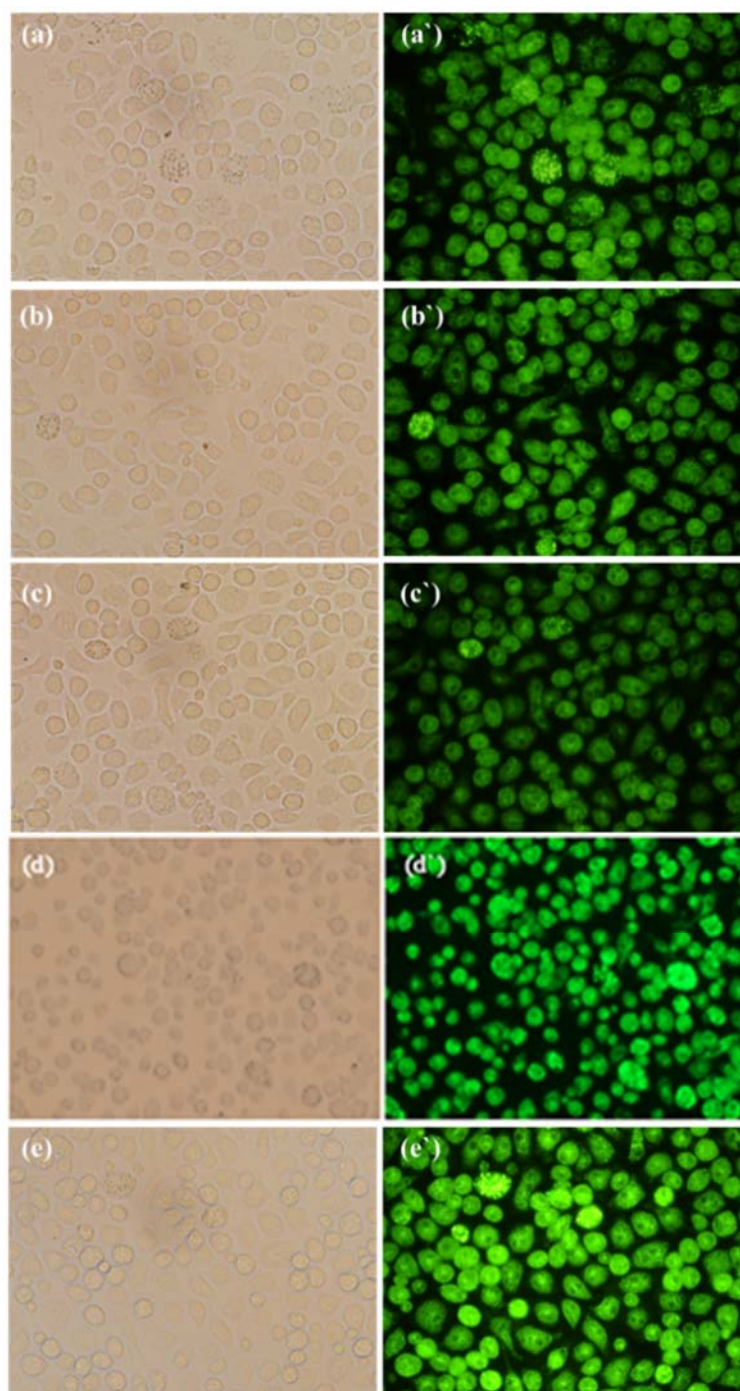


Figure 8 Fluorescence microscopic images demonstrating the micelles/nanoparticles phagocytized by MPM for 2 h. The cells were incubated in different micelles/nanoparticles solutions with the same concentration of loaded 6-coumarin. (a, a'): PMPC₁₁-*b*-PBS-*b*-PMPC₁₁; (b, b'): PMPC₂₅-*b*-PBS-*b*-PMPC₂₅; (c, c'): PMPC₆₄-*b*-PBS-*b*-PMPC₆₄; (d, d'): PLA Nps and (e, e'): PDS55. Light microscopic

images (a, b, c, d and e) and fluorescence microscopic images (a', b', c', d' and e') were taken at the same illumination and exposure time, respectively.

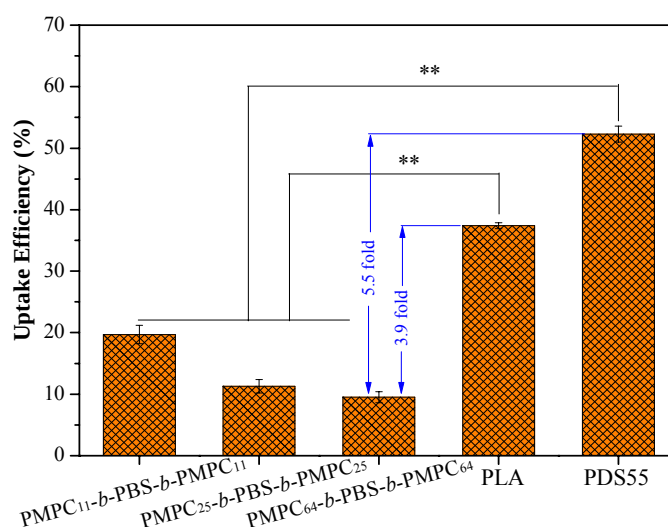


Figure 9 Quantitative assay of phagocytic uptake efficiency of micelles by mouse peritoneal macrophages. PDS55 micelles and PLA nanoparticles are used as the positively charged and negatively charged control samples, respectively. The data presented are mean $\pm$ SD from four separate experiments.

CONCLUSIONS

We have demonstrated that triblock copolymer of PMPC-*b*-PBS-*b*-PMPC with controlled molecular weight and functionality can be conveniently prepared by the combination of polycondensation and atom transfer radical polymerization. Meanwhile, PMPC-*b*-PBS-*b*-PMPC formed nano-sized micelles in water with phosphorylcholine shell structure. DOX was incorporated into the micelles and released at an accelerated rate under acidic pH value. The polymeric micelles were non-toxicity to L929 cells and low-toxicity to Hela cells. In addition, after being loaded with DOX, the solubility of polymeric micelle against DOX improved its pesticide effect in *in-vitro* viability test. Importantly, to our knowledge, this is a first report on the synthesis of biodegradable polyesters PBS modified by PMPC. Moreover, there were few reports about drug carrier micelles with PBS as hydrophobic core. In conclusion, the formation of PMPC-*b*-PBS-*b*-PMPC micelles provided great potential for

fabricating more effective drug carriers that could minimize the clearance of the reticuloendothelial system and prolong the circulation time. The result indicates that PBS can be an ideal candidate for drug delivery system.

ACKNOWLEDGEMENTS

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Tables

Table 1 ATRP results and molecular weight characterization of copolymer PMPC-*b*-PBS-*b*-PMPC

Table 2 The properties of PMPC-*b*-PBS-*b*-PMPC micelles with and without DOX. Each value was expressed as mean $\pm$ SD from three independent measurements.

Figure Captions

Scheme 1 Schematic illustration of the preparation of DOX-loaded PMPC-*b*-PBS-*b*-PMPC micelles and the subsequent release of DOX from micelles in tumor and normal vessels.

Scheme 2 Synthetic routes of the triblock copolymer of PMPC-*b*-PBS-*b*-PMPC

Figure 1 ^1H NMR spectra recorded at 20 °C for (A) HO-PBS-OH in CDCl_3 ; (B) Br-PBS-Br in CDCl_3 ; (C) $\text{PMPC}_{11}\text{-}b\text{-PBS-}b\text{-PMPC}_{11}$ in a 1:1 $\text{CDCl}_3/\text{CD}_3\text{OD}$ mixture (which is a good solvent for both blocks); (D) $\text{PMPC}_{11}\text{-}b\text{-PBS-}b\text{-PMPC}_{11}$ polymeric micelles in D_2O .

Figure 2 DSC thermograms for the polymers of HO-PBS-OH (A), $\text{PMPC}_{11}\text{-}b\text{-PBS-}b\text{-PMPC}_{11}$ (B), $\text{PMPC}_{25}\text{-}b\text{-PBS-}b\text{-PMPC}_{25}$ (C) and $\text{PMPC}_{64}\text{-}b\text{-PBS-}b\text{-PMPC}_{64}$ (D).

Figure 3 Size distribution profiles of $\text{PMPC}_{11}\text{-}b\text{-PBS-}b\text{-PMPC}_{11}$ micelles in distilled water determined by TEM (A), and DLS measurement (B).

Figure 4 *In vitro* release profiles of DOX loaded $\text{PMPC-}b\text{-PBS-}b\text{-PMPC}$ micelles in phosphate buffer pH 7.4 and pH 5.5 at 37 °C, respectively.

Figure 5 *In vitro* cytotoxicities of $\text{PMPC-}b\text{-PBS-}b\text{-PMPC}$ micelles on L929 (A) and Hela (B) cells were determined by MTT assay after 24 h incubation at 37 °C. Cell viability (%) was determined based on the ratio of optical density of treated cells to that of the untreated cells. Data were expressed as mean $\pm$ SD, n=4.

Figure 6 *In vitro* viabilities of free Dox and Dox-loaded $\text{PMPC}_{64}\text{-}b\text{-PBS-}b\text{-PMPC}_{64}$ micelles on Hela cells determined by the MTT assay after 24 h incubation at 37 °C. All the micelles were loaded roughly with 12~13% of DOX, and the same amount of free DOX were added as in the control samples. Cell viability (%) was determined based on the ratio of optical density of treated cells to that of the untreated cells. Data were expressed as mean $\pm$ SD, n=4.

Figure 7 The flow cytometry histograms (A), and the mean fluorescence intensities (B) of cellular uptake of HeLa cells after incubation with free DOX or DOX-loaded $\text{PMPC-}b\text{-PBS-}b\text{-PMPC}$ micelles for 2 h at a DOX concentration of $8\text{ }\mu\text{g mL}^{-1}$. Data represent mean $\pm$ SD (n = 3).

Figure 8 Fluorescence microscopic images demonstrating the micelles/nanoparticles phagocytized by MPM for 2 h. The cells were incubated in different micelles/nanoparticles solutions with the same concentration of loaded 6-coumarin. (a, a'): $\text{PMPC}_{11}\text{-}b\text{-PBS-}b\text{-PMPC}_{11}$; (b, b'): $\text{PMPC}_{25}\text{-}b\text{-PBS-}b\text{-PMPC}_{25}$; (c, c'): $\text{PMPC}_{64}\text{-}b\text{-PBS-}b\text{-PMPC}_{64}$; (d, d'): PLA Nps and (e, e'): PDS55. Light microscopic

images (a, b, c, d and e) and fluorescence microscopic images (a', b', c', d' and e') were taken at the same illumination and exposure time, respectively.

Figure 9 Quantitative assay of phagocytic uptake efficiency of micelles by mouse peritoneal macrophages. PDS55 micelles and PLA nanoparticles are used as the positively charged and negatively charged control samples, respectively. The data presented are mean $\pm$ SD from four separate experiments.