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Synthesis and characterization of star-shaped block copolymer of poly-(ε-caprolactone) and poly(ethyl ethylene phosphate) as drug carrier

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ABSTRACT

A series of novel 4-arm biodegradable star block copolymers of poly(ε-caprolactone) and poly(ethyl ethylene phosphate) were synthesized via ring-opening polymerization of 2-ethoxy-2-oxo-1,3,2-dioxaphospholane using hydroxyl terminated 4-arm star-shaped poly(ε-caprolactone) and stannous octoate co-initiation system. Gel permeation chromatography (GPC), NMR and FT-IR were used to demonstrate the structure and analyze their compositions. The self-assembly behavior of these star-shaped copolymers in aqueous solution was studied by ^{1}H NMR and fluorescence technique, and the results indicated those copolymers formed nanoparticles in aqueous solution with hydrophobic poly(ε-caprolactone) core and hydrophilic poly(ethyl ethylene phosphate) shell. The critical micelle concentration was relative to the length of poly(ε-caprolactone) and poly(ethyl ethylene phosphate) block. Paclitaxel was encapsulated in the micelles and the release behavior demonstrated that a longer hydrophobic block resulted in slightly slower release rate from the micelles. These copolymer micelles were biocompatible and potential as drug-delivery vehicles for pharmaceutical application.

1. Introduction

Biodegradable and biocompatible amphiphilic copolymers used as carriers for drug delivery have gained increasing interest in the past decade [1–5]. The amphiphilic copolymers are able to self-assemble into polymeric micellar nanoparticles in aqueous solution with a hydrophobic core that are stabilized by a hydrophilic shell [6]. The hydrophobic core is mainly composed of aliphatic polyester, typically poly(ε-caprolactone) (PCL), poly(lactide) (PLA), poly(hydroxybutyrate) (PHB) or their copolymers, working as the sustained release reservoir of insoluble molecules or unstable agents [7–12]. The hydrophilic shell, however, is particularly composed of polyethylene glycol (PEG), which is believed to prevent nanoparticle aggregations and protein adhesion, and prolong circulation of nanoparticles [13].

Amphiphilic star-shaped polymers with a few or a large number of linear hydrophilic arms are attractive because it may overcome or partially overcome the problem of thermodynamic instability associated with micelles assembled from linear block copolymers following systemic injection [14]. Owing to their particular architecture, star-shaped polymers are expected to exhibit different properties compared to their linear counterparts. For example, star-shaped polymers are superior in host–guest interactions with small organic molecules to the linear counterparts [15]. Generally, star-shaped polymers can be synthesized by the "arm-first" [16] or the "core-first" [17] strategy while the latter is more popular due to the better control on molecular structure [18]. Using the "core-first" method, Hedrick et al. have synthesized star polylactone using hexahydroxy-functional compound as the initiator and stannous octoate as the catalyst [19]. Many other star-shaped block copolymers such as that of PCL and PLA [20,21], PCL and PEG [22], PLA and thermosensitive poly(*N*-isopropylacrylamide) [20] have been synthesized using the "core-first" method. It has also been demonstrated that amphiphilic star-shaped copolymers provide opportunities for novel drug delivery system development [21,22].

We reported here 4-arm star-shaped amphiphilic copolymers composed of PCL as hydrophobic segments while polyphosphoester (PPE) as the hydrophilic component instead of the traditional polyethylene glycol. As a class of biodegradable polymers with repeated phosphoester linkage in the backbone, PPE has been used for many biomedical applications including in drug, gene delivery, and tissue engineering due to its favorable biocompatibility and biodegradability [23–25]. In our pervious work, we have reported that linear triblock copolymers of poly(ethyl ethylene phosphate) (PEEP) and PCL self-assemble into core-shell structured micellar nanoparticles, which are biodegradable and biocompatible. Moreover, the drug release profile can be adjusted by the composition of

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polymers [26]. In this study, we synthesized a series of novel 4-arm biodegradable star block copolymers of PCL and PEEP via ring-opening polymerization of 2-ethoxy-2-oxo-1,3,2-dioxaphospholane (EEP) using hydroxyl terminated 4-arm star-shaped PCL and stannous octoate co-initiation system. The self-assembly behavior and potential for drug delivery were also studied.

2. Experimental

2.1. Materials

Pentaerythritol (PTOL, Sinopharm Chemical Reagent Co., Ltd., China) was sublimed under vacuum at 200 °C. ε-Caprolactone (CL) (Acros Organics, 99%) was dried over calcium hydride for 48 h at room temperature, followed by distillation under reduced pressure just before use. Stannous octoate ($Sn(Oct)_2$, Sinopharm Chemical Reagent Co., Ltd., China), was purified according to a method described in the literature [27]. 2-Ethoxy-2-oxo-1,3,2-dioxaphospholane (EEP, also known as ethyl ethylene phosphate) was synthesized as previously described in the literature [28] and purified with two successive vacuum distillations. Tetrahydrofuran (THF) was refluxed over potassium–sodium alloy under N_2 atmosphere and distilled out just before use. Spectra/Por® membranes (Spectrum Laboratories, Inc., Rancho Dominguez, CA) were employed for dialysis. Paclitaxel (PTX) and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) were obtained from Sigma Chemical Co. Sodium dodecyl sulfate (SDS) was obtained from Shanghai Sangon Biological Engineering Technology Co., Ltd. Pyrene (Acros Organics) and all other solvents and reagents were used as-received.

2.2. Synthesis of 4-arm star-shaped poly(ε-caprolactone) (ssPCL)

ssPCL was synthesized through ring-opening polymerization (ROP) of ε-caprolactone in bulk using pentaerythritol as an initiator and $Sn(Oct)_2$ as the catalyst. Typically, CL (3.5×10^{-2} mol, 3.990 g), pentaerythritol (1×10^{-3} mol, 0.136 g), and $Sn(Oct)_2$ (5×10^{-5} mol, 0.020 g) were added into a fresh flamed and nitrogen purged round-bottomed flask in a glove box with H_2O and O_2 contents less than 0.1 ppm. The mixture was maintained at 120 °C for 4 h. The product was dissolved in THF (10 mL) and precipitated in cold ethyl ether twice. The precipitate was collected and dried under vacuum to a constant weight at room temperature.

2.3. Synthesis of 4-arm star-shaped block copolymer of poly-(ε-caprolactone) and poly(ethyl ethylene phosphate) (ssPCL–PEEP)

Block copolymerization was carried out in THF at 25 °C using ssPCL as macroinitiator and $Sn(Oct)_2$ as the catalyst. In a typical polymerization, $ssPCL_{33}$ (star-shaped polymer of PCL with the chain length of 33) (1×10^{-4} mol, 0.0376 g), EEP (0.608 g, 4×10^{-3} mol), and $Sn(Oct)_2$ (5×10^{-5} mol, 0.020 g) were dissolved in anhydrous THF (5 mL) in a fresh flamed and nitrogen purged round-bottomed flask at 25 °C. The mixture was further reacted for 1 h, concentrated and precipitated in cold ethyl ether/methanol (10/1, v/v) twice at 15 °C. The product was filtrated and dried under vacuum to a constant weight at room temperature. The yield was approximately 85%.

2.4. Characterization of polymers

Bruker AV300 NMR spectrometer operating at 300 MHz was used for ^1H and ^{13}C NMR spectra to determine the structure and composition of all the compounds synthesized in this work with either $CDCl_3$ or D_2O as solvent. FT-IR spectra of polymers were measured on a Bruker Vector 22 Fourier Transform Infrared Spectrometer at wavenumbers 500–4000 cm^{-1} with a resolution of 2 cm^{-1} using the KBr disk method. Number and weight average molecular weights (M_n and M_w) and molecular weight distributions (polydispersity index, $PDI = M_w/M_n$) were determined by gel permeation chromatography (GPC) measurements on a Waters GPC system, which was equipped with a Waters 1515 HPLC solvent pump, a Waters 2414 refractive index detector, and four Waters styragel high resolution columns (HR4, HR2, HR1, HR0.5, effective molecular weight range 5000–500,000, 500–20,000, 100–5000, and 0–1000, respectively). Chloroform (HPLC grade, J.T. Baker, stabilized with 0.75% ethanol) was used as mobile phase at 40 °C, delivered at a flow rate of 1.0 mL min^{-1}. Monodispersed polystyrene standards from Waters Co. with a molecular weight range 1310–5.51×10^4 were used to generate the calibration curve. Polymer samples were dissolved in chloroform at 4 mg mL^{-1} and injected into the system.

2.5. Preparation of micelles

Micelles were prepared by the dialysis method. Briefly, 10 mg of ssPCL–PEEP copolymer was dissolved in 1 mL of THF and the solution was allowed to stand at room temperature for 2 h. Then, to this solution, was added 10 mL of Milli-Q ultrapurified water at 60 mL h^{-1} under gentle stirring. After standing for 3 h at room temperature, THF was removed by dialysis against water for 24 h to obtain the micelles.

2.6. Loading paclitaxel into ssPCL–PEEP micelles

Typically, a block copolymer (2 mg) solution in 1.0 mL of THF was mixed with 0.2 mg of paclitaxel. The mixture was allowed to stand at room temperature for 2 h. Then, 10 mL of ultrapurified water was added dropwise to this solution at the speed of 60 mL h^{-1} under gentle stirring. The mixture was stirred at room temperature for 3 h and the organic solvent was removed by dialysis against water for 24 h to obtain the micelles. The solution was filtered through 0.45 μm filter and freeze-dried. To obtain the amount of PTX loaded into micelles, we first dissolved paclitaxel-loaded micelles with acetonitrile–water (80:20, v/v) to get the complete release of the PTX, and then use HPLC to determine the amount of PTX loaded into micelles as described below. The drug loading content (DLC %) and efficiency (DLE %) were calculated by the following equations:

$$\mathrm{DLC}\% = \frac{\text{amount of PTX in micelle}}{\text{amount of PTX} - \text{loaded micelle}} \times 100\%$$

$$\mathrm{DLE}\% = \frac{\text{amount of PTX in micelle}}{\text{amount of PTX used for micelle preparation}} \times 100\%$$

2.7. Paclitaxel release from micelles

In vitro release profiles of paclitaxel from micelles were investigated in phosphate buffered saline (PBS, 0.01 mol L^{-1}, pH 7.4). Micelles (3 mL) was introduced into a dialysis membrane tubing (Spectra/Por®, Float-A-Lyzer, MWCO 25,000) and incubated in 20 mL of PBS at 37 °C with stirring. At predetermined intervals, buffer was taken out and an equal volume of fresh buffer was added. The concentration of paclitaxel in the solution was measured by HPLC as described below.

2.8. Fluorescence measurements

To estimate the critical micelle concentrations (CMCs) of the micelles, pyrene was used as a hydrophobic fluorescence probe. A

predetermined amount of pyrene in acetone was added into a series of ampules, and the acetone was then removed first by gently flowing N_2 and then by vacuum. A predetermined volume of copolymer solutions and ultrapurified water were added into the ampules consecutively to get solutions of different micelle concentrations ranging from 1.22×10^{-4} to 0.25 g L^{-1}, while the concentration of pyrene in each flask was fixed at 6.0×10^{-7} mol L^{-1}, slightly lower than the saturation solubility of pyrene in water. The excitation spectra (300–360 nm) were recorded on a Shimadzu RF-5301PC spectrofluorophotometer with an emission wavelength of 390 nm. The excitation and emission bandwidths were set at 5 nm.

2.9. Particle size and zeta potential measurements

Particle size and size distribution measurements in aqueous solution were done using dynamic light scattering (DLS) carried out on a Malvern Zetasizer Nano ZS90 equipped with a He–Ne laser (633 nm), and 90° collecting optics. Malvern Dispersion Technology Software 4.20 was applied for data analysis. All samples were prepared in aqueous solution at a concentration of 0.1 g L^{-1} and filtered through Millipore 0.45 μm filter prior to measurements. All measurements were carried out at 25 °C. The zeta potential measurements were performed using an aqueous dip cell in automatic mode using Malvern Zetasizer Nano ZS90.

2.10. MTT assay

The relative cytotoxicity of micelles was assessed with a methyl tetrazolium (MTT) viability assay against HEK293 cells from ATCC. The cells were seeded in 96-well plates at 20,000 cells per well in 100 μL of complete DMEM containing 10% fetal bovine serum, supplemented with 50 U mL^{-1} penicillin and 50 U mL^{-1} streptomycin, and incubated at 37 °C in 5% CO_2 atmosphere for 24 h, followed by removing culture medium and adding micelle solutions (100 μL in complete DMEM medium) at different concentrations (0–1 g L^{-1}). After 72 h incubation, 25 μL of MTT stock solution (5 g L^{-1} in PBS) was added to each well to achieve a final concentration of 1 g L^{-1}, with the exception of the wells as blank, to which 25 μL of PBS was added. After incubation for an additional 2 h, 100 μL of the extraction buffer (20% SDS in 50% DMF, pH 4.7, prepared at 37 °C) was added to the wells and incubated overnight at 37 °C. The solution was mixed, and the absorbance of the solution was measured at 570 nm using a Bio-Rad 680 microplate reader. The cell viability was normalized to that of HEK293 cells cultured in the culture medium without polymer micelle.

2.11. HPLC analysis of paclitaxel

The amount of PTX in the micelle or released to the solution was analyzed by HPLC, which was performed on Waters HPLC system consisting of Waters 1525 binary pump, Waters 2487 2-channel UV–vis detector, 1500 column heater and a Symmetry® C18 column. Acetonitrile–water (50:50, v/v) was used as the mobile phase at 30 °C with a flow rate of 1.0 mL min^{-1}. UV–vis Detector was set at 227 nm and linked to Breeze software for data analysis. Linear calibration curves for concentrations in the range of 0.098–100 μg mL^{-1} were constructed using the peak areas by linear regression analysis. The regression equation was calculated as $y = 36{,}866.5x + 14{,}666.9$ ($R^2 = 0.9995$). The concentrations of paclitaxel were determined by comparing the peak area with the stand curve.

3. Results and discussion

3.1. Synthesis and characterization of ssPCL–PEEP block copolymers

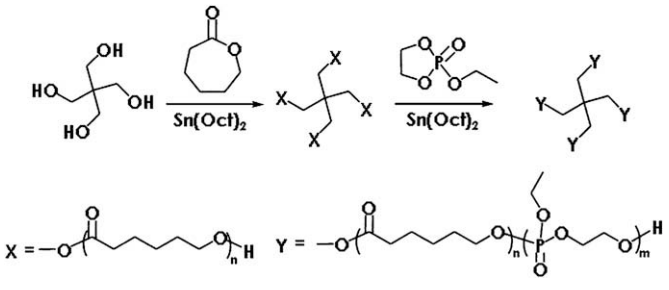
ssPCL–PEEP copolymers were prepared following a two-step synthetic procedure using the "core-first" approach. Accordingly, ssPCL was first synthesized using a tetrafunctional initiator, and the hydroxyl end groups of PCL chains subsequently initiated ring-opening polymerization of EEP as shown in Scheme 1. With the catalysis of $Sn(Oct)_2$, ssPCL bearing four hydroxyl end groups were obtained by ring-opening polymerization of CL in bulk using pentaerythritol as the initiator. The total numbers of CL units in four arms were varied by adjusting the feeding ratio of initiator to CL. As summarized in Table 1, DP (degree of polymerization) of CL was close to the feeding ratio of CL to hydroxyl group of pentaerythritol, which was calculated based on the integration ratio of peak intensities between methylene proton besides carbonyl group (–OCOCH_2CH$_2$CH$_2$CH$_2$CH$_2$O–, 2.34 ppm) and methylene protons beside the oxygen (–OCOCH$_2$CH$_2$CH$_2$CH$_2$CH_2OH, 3.63 ppm) (data not shown).

The molecular weight distribution of ssPCL was around 1.2–1.30 which was determined by gel permeation chromatography. It is worth noting that M_n values obtained by GPC measurements were higher than those calculated from ^{1}H NMR due to the structural difference between the resultant polymers and monodispersed polystyrene standards which were used to generate the calibration curve in GPC analysis. This phenomenon was also observed by many other groups in GPC analysis of PCL and its copolymers [27,29]. In this study, ssPCL with 33, 44 and 69 total CL units were used as the macroinitiator for further polymerization of EEP.

In the second step, ssPCL was used as the initiator for ROP of EEP at 25 °C in THF in the presence of $Sn(Oct)_2$ as the catalyst. The straightforward proof of formation of the block copolymer formation was provided by comparison of the GPC spectra of ssPCL$_{69}$ and ssPCL$_{69}$–PEEP$_{56}$ shown in Fig. 1. The unimodal peak of ssPCL$_{69}$–PEEP$_{56}$ with decreased retention time, corresponding to higher molecular weights, demonstrated the formation of block polymer. The PDIs of those star-shaped copolymers were from 1.45 to 1.70, the high PDI obtained may be due to the fact that multiarm star macroinitiator ssPCL possess more initiating sites than the linear PCL, and cause the incomplete and disequilibrium initiation [30,31].

The basic data of the resultant star-shaped block copolymers, including molecular weights, polydispersity indexes and compositions, are summarized in Table 2. The nomenclature used for the star-shaped block copolymers displays the degree of polymerization (DP). For example, ssPCL$_{33}$–PEEP$_{53}$ represents star-shaped block copolymer composed of total 33 CL units and 53 EEP units.

Fig. 2A showed a representative ^{1}H NMR spectrum of ssPCL$_{69}$–PEEP$_{56}$. Resonances at δ 4.18 (c), and 4.26 (a, b) were assigned to pendent methylene (–OCH_2CH$_3$) protons and methylene protons (–POCH_2CH_2O–) from PEEP backbone, respectively, which were characteristic signals of PEEP block. In addition, signal at 3.63 ppm assigned to terminal methylene protons of ssPCL completely



Scheme 1. Synthesis pathway of ssPCL–PEEP copolymers.

Table 1
Results of ring-opening polymerization of ε-caprolactone initiated with pentaerythritol with $Sn(Oct)_2$ as catalyst

Sample	Total CL units of ssPCL	M_n[a]	M_n[b]	PDI[a]
$ssPCL_{33}$	33	5173	3770	1.22
$ssPCL_{44}$	44	6955	5020	1.22
$ssPCL_{69}$	69	11,285	7880	1.30

[a] Determined by GPC.
[b] Determined by ^1H NMR spectroscopy.

Table 2
Results of block copolymerization of EEP initiated with ssPCL with $Sn(Oct)_2$ as catalyst

Sample	Feeding ratio of EEP to CL units of ssPCL	Grafting of EEP (%)	M_n[a]	M_n[b]	PDI[a]
$ssPCL_{33}$–$PEEP_{25}$	30:33	83.3	9980	7570	1.68
$ssPCL_{33}$–$PEEP_{35}$	40:33	87.5	10,790	9080	1.70
$ssPCL_{33}$–$PEEP_{53}$	60:33	88.3	13,720	11,820	1.45
$ssPCL_{33}$–$PEEP_{69}$	75:33	92.0	14,900	12,890	1.47
$ssPCL_{44}$–$PEEP_{32}$	40:44	80.0	12,300	9,890	1.46
$ssPCL_{69}$–$PEEP_{35}$	40:69	87.5	15,240	13,200	1.48
$ssPCL_{69}$–$PEEP_{56}$	60:69	93.3	18,490	16,390	1.58

[a] Determined by GPC.
[b] Determined by ^1H NMR.

disappeared after polymerization of EEP. Instead, signal at 3.8 ppm, which is characteristic and should be assigned to methylene protons conjoint to hydroxyl end groups of PEEP appeared, indicating that ssPCL macroinitiators were completely involved in the copolymerization. The degree of polymerization (DP) of PEEP was calculated from the integrated peak area of 4.18 and 4.26 ppm (6H) assigned to methylene groups of PEEP block, by the integrated peak of the triplets at 2.30 ppm ($OCOCH_2CH_2CH_2CH_2CH_2O$), assigned to the methylene group of PCL block.

^{13}C NMR spectrum of the copolymer also attested the block structure of the copolymer as illustrated in Fig. 2B. A signal peak at 173.6 ppm in carbonyl region is in agreement with the block structure of the copolymer, as reported previously [21]. In the FT-IR spectra (Fig. 3), absorbance at 1730 and 1045 cm^{-1} are characteristic absorptions of the C═O stretching and C–O stretching due to the presence of PCL block. Absorptions of asymmetrical and symmetrical P═O stretchings occurred at 1260 and 1160 cm^{-1} respectively, while P–O–C stretching appeared at 984 cm^{-1} demonstrating the presence of PEEP block. The presence of strong hydroxyl (–OH) stretching at around 3439 cm^{-1} indicates that the polymer is hydroxyl terminated.

3.2. Micellization of ssPCL–PEEP block copolymers

It is well known that amphiphilic block copolymers self assemble into aggregates in a selective solvent. Such aggregates including micelles and vesicles have been widely studied as drug carriers [9,10]. In our previous studies, we have reported that block copolymer of hydrophilic PEEP and hydrophobic PCL reversibly self-assemble into aggregates [26]. In this study, we dissolved ssPCL–PEEP in tetrahydrofuran and mixed the solution with water to induce microphase separation of PCL and PEEP blocks, followed by removing the organic solvent THF by dialysis. To demonstrate the formation of self-assembled aggregates, lyophilized sample was resuspended in D_2O and the ^1H NMR was measured. As shown in Fig. 4A, the typical ^1H NMR spectrum of micelles in D_2O showed that signals assigned to protons of PCL block were significantly suppressed, while signals at 4.26, 4.18 and 1.30 ppm assigned to protons of PEEP block were still prominent, indicating the limited molecular motion of PCL block surrounded by the solvated PEEP segments.

The micelle formation of the copolymers was also confirmed by fluorescence technique using pyrene as a probe. Fig. 4B shows the fluorescence excitation spectra of pyrene in $ssPCL_{33}$–$PEEP_{35}$ micelles at different concentrations. It is obvious that fluorescence intensity increases with increasing copolymer concentration, due to transfer of pyrene into a hydrophobic environment. A red shift of (0, 0) absorption band from 336 to 339 nm was observed when the concentration of copolymer was increased from 1.22×10^{-4} to 0.25 g L^{-1}. This red shift results from the transfer of pyrene molecules from a water environment to the hydrophobic micellar core, and thus provides information on the location of the pyrene probe in the system, in fact, indicating the formation of micelles [23]. On the other hand, from the plot of fluorescence intensity ratio of I_{339}/I_{336} versus log C of the copolymer (Fig. 5), the CMC values were obtained, taken as the intersection of the tangents to the horizontal line of intensity ratio with relatively constant values and the diagonal line with rapid increased intensity ratio.

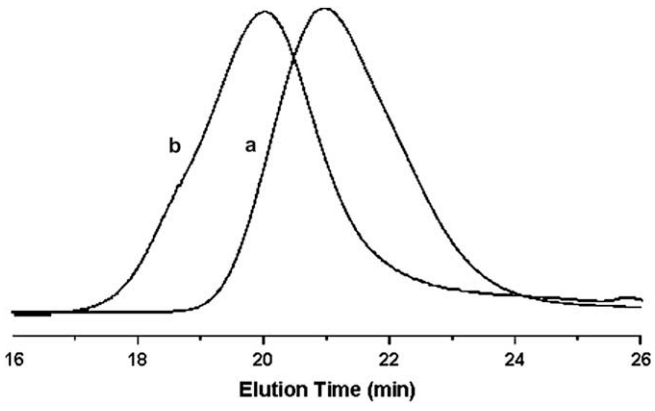


Fig. 1. GPC spectra of $ssPCL_{69}$ (a) and $ssPCL_{69}$–$PEEP_{56}$ (b).

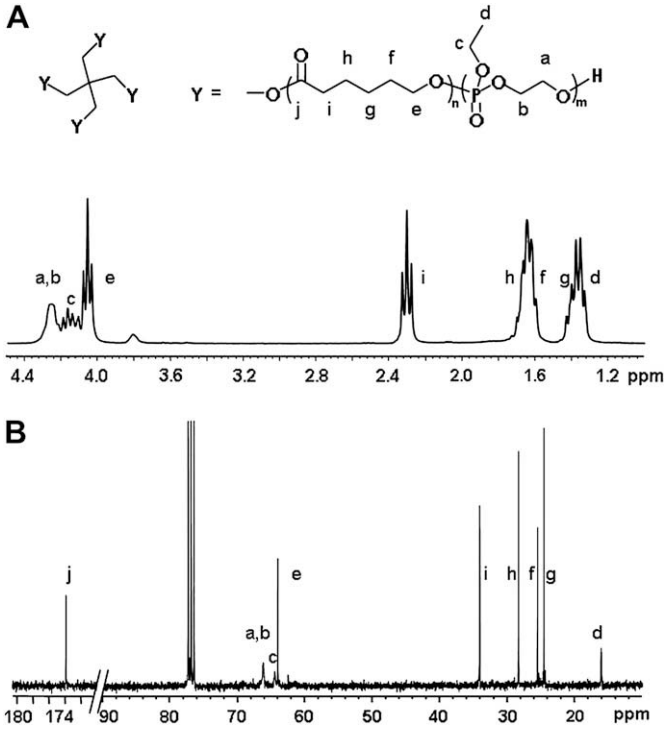


Fig. 2. NMR spectra of typical ssPCL–PEEP copolymer: (A) ^1H, (B) ^{13}C NMR (in $CDCl_3$).

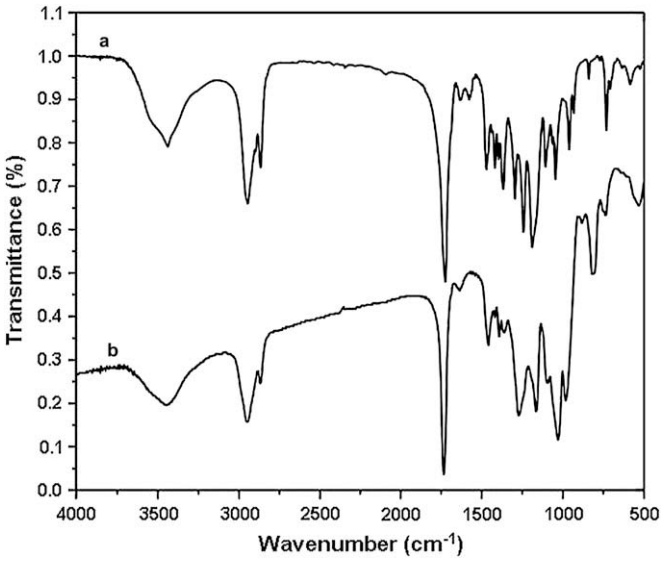


Fig. 3. FT-IR spectra of (a) $ssPCL_{44}$ and (b) $ssPCL_{44}$–$PEEP_{32}$.

CMC values are listed in Table 3. It was observed that CMC values were affected by the composition of block copolymers. For example, longer PEEP chain resulted in higher CMC value. When DP of PCL was fixed at 33, CMC value increased with increasing DP of PEEP from 25 to 69. When the DP of PEEP was similar from 32 to 35, but the DP of PCL was varied from 33 to 69 units, the CMC value slightly changed, indicating that the hydrophobic length plays less important role on the stability of these micelles.

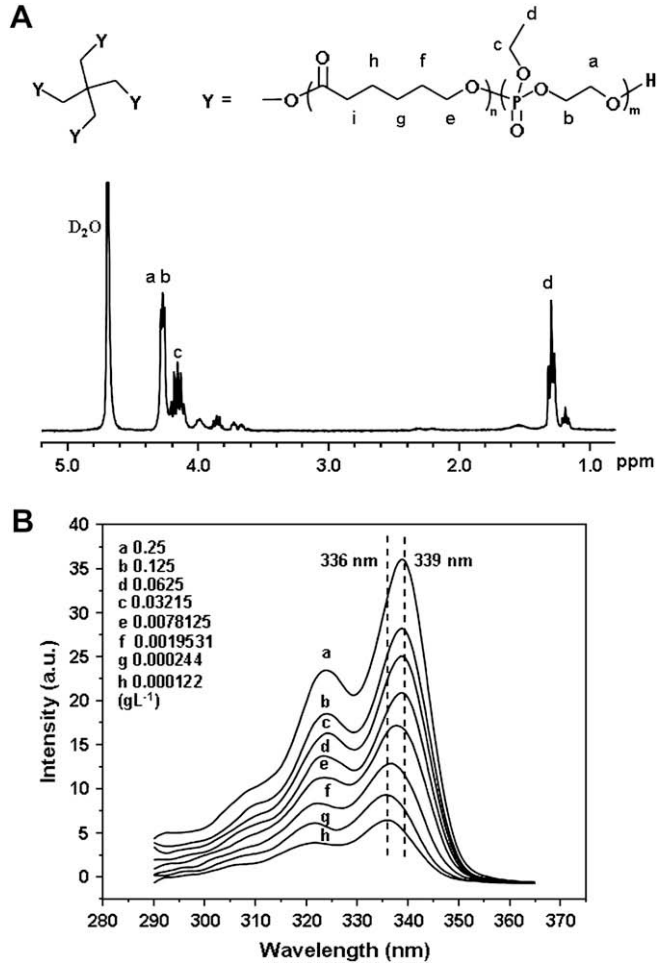


Fig. 4. (A) ^1H NMR spectrum of $ssPCL_{33}$–$PEEP_{35}$ copolymer micelle in D_2O; (B) excitation spectra of pyrene in aqueous solution of $ssPCL_{33}$–$PEEP_{35}$ at various concentrations ($\lambda_{em} = 390$ nm).

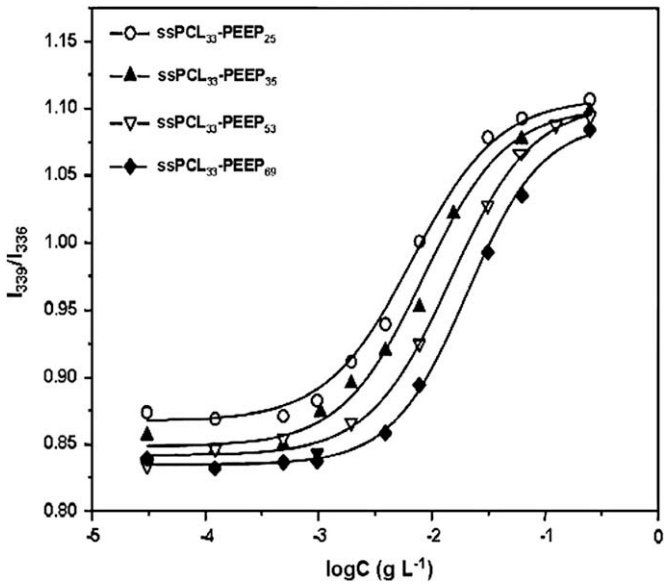


Fig. 5. Intensity ratio (I_{339}/I_{336}) as a function of concentration of ssPCL–PEEP with different DP of PEEP.

Micelles size and their distribution were measured by dynamic light scattering and summarized in Table 3. The size of micelles was around 45–110 nm and increased after drug loading. It is worth noting that $ssPCL_{33}$–$PEEP_{25}$ formed larger particles with an average diameter of 108 nm, which is possibly due to the formation of vesicular structure in aqueous solution. The copolymer micelles were negatively charged with zeta potential around −25 mV, likely attributed to polarization effect due to the presence of pentavalent phosphorus heteroatoms of PEEP block.

3.3. In vitro *cytotoxicity*

The cytotoxicity of block copolymer to HEK293 cells was evaluated using MTT assay. As an example, Fig. 6 showed the cell viability treated with $ssPCL_{69}$–$PEEP_{35}$ at different concentrations for 72 h, which was compared with cells treated with sodium dodecyl sulfate. It was observed that more than 80% cells cultured with $ssPCL_{69}$–$PEEP_{35}$ remained viable when the concentration was up to 1.0 g L^{-1}, and at lower concentration, $ssPCL_{69}$–$PEEP_{35}$ did not show any cytotoxicity, suggesting the good cell compatibility of ssPCL–PEEP to HEK293 cells. In our previous study, we also observed that linear PCL–PEEP showed similar cytocompatibility

Table 3
Effect of copolymer composition on CMC values, drug loading contents and drug loading efficiency, as well as the size and size distribution of the micelles produced

Sample	CMC (g L^{-1})	Diameter of micelles before PTX loading (nm)	Diameter of micelles after PTX loading (nm)	DLC (%)	DLE (%)
$ssPCL_{33}$–$PEEP_{25}$	1.10×10^{-3}	108.0 ± 4.0	225.0 ± 8.9	1.62	16.19
$ssPCL_{33}$–$PEEP_{35}$	1.58×10^{-3}	45.1 ± 2.3	114.0 ± 3.1	2.64	26.38
$ssPCL_{33}$–$PEEP_{53}$	2.38×10^{-3}	76.2 ± 2.4	95.4 ± 8.7	3.46	34.59
$ssPCL_{33}$–$PEEP_{69}$	3.87×10^{-3}	66.8 ± 4.0	80.6 ± 6.0	2.09	20.88
$ssPCL_{44}$–$PEEP_{32}$	6.93×10^{-4}	37.4 ± 0.1	107.0 ± 1.7	2.79	27.86
$ssPCL_{69}$–$PEEP_{35}$	5.74×10^{-4}	61.4 ± 0.7	119.0 ± 0.6	2.52	25.08
$ssPCL_{69}$–$PEEP_{56}$	1.03×10^{-3}	70.6 ± 0.5	90.5 ± 0.4	2.63	26.28

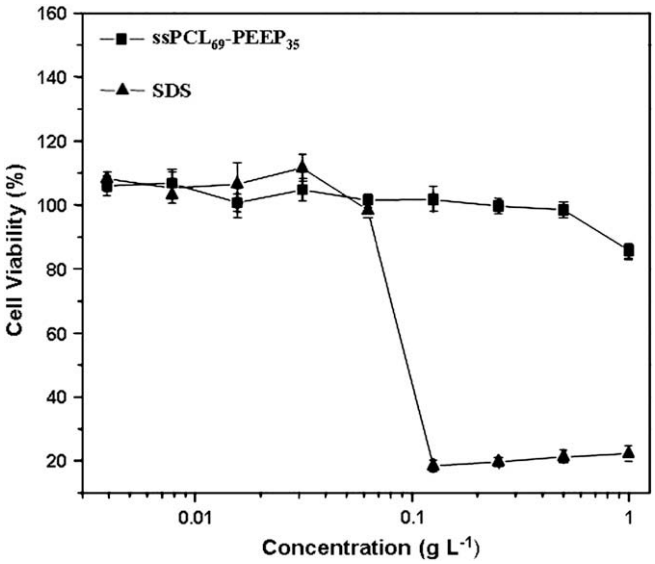


Fig. 6. Cytotoxicity of $ssPCL_{69}$–$PEEP_{35}$ to HEK293 cells.

and about 90% cells remained viable when HEK293 cells were treated with linear PCL–PEEP at 1.0 mg mL^{-1} using the same protocol [26].

3.4. Paclitaxel loading and release

PTX has shown significant activity against a wide range of solid tumors, especially drug-resistant ovarian cancer, metastatic breast cancer, and non-small-cell lung cancer [32]. Due to its hydrophobic property, a lot of vehicles were designed as the carriers of PTX. In this work, using a dialysis method, we successfully loaded paclitaxel into the hydrophobic core of ssPCL–PEEP micelles. The particle size after PTX loading is given in Table 3. The drug loading contents (DLC%) were between 1.6 and 3.5%, depending on the composition of copolymers, while the drug loading efficiencies were between 16 and 35%, which is higher than that using the linear counterparts (around 15%).

The drug release behavior was demonstrated by plotting the accumulative release percentages of drug versus time in Fig. 7, at the beginning of drug release, a burst release of PTX was observed for the copolymers, primarily attributed to the diffusion of PTX located close to the surface of particles, a sustained release lasting for about 2 weeks from these micelles was observed, which exhibited a slower drug release rate compared to the linear counterpart. About 45–85% of the initial loading amount was eventually released from these micelles in the test duration. Polymer structure was found to be correlative to the drug release kinetics. For example, $ssPCL_{33}$–$PEEP_{69}$ micelles gave a faster release, while PTX release from $ssPCL_{69}$–$PEEP_{56}$ micelles was slower. It is also observed that about 25–55% of loaded PTX were not released from the micelles in the test duration, which may be associated with the hydrophobic core of micelles, as observed by other groups [33].

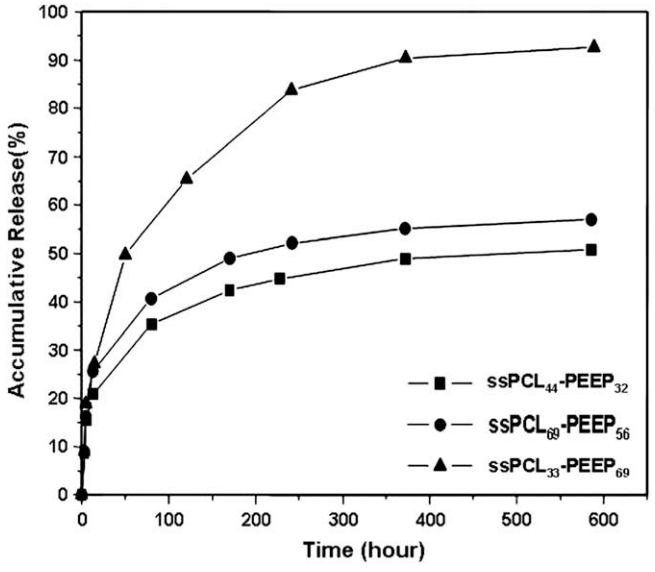


Fig. 7. Release profile of paclitaxel from ssPCL–PEEP micelles in PBS at 37 °C.

4. Conclusion

A series of novel biodegradable star block copolymers with four arms composed of hydrophobic PCL and hydrophilic PEEP were successfully synthesized using a "core-first" strategy. The copolymers were obtained by polymerization of CL, followed by ring-opening polymerization of EEP in the presence of $Sn(Oct)_2$. The block length can be well controlled though changing the feed ratio of monomers in ring-opening copolymerization. According to the results of ^1H NMR and fluorescence measurements, these star-shaped block copolymers can self-assemble into micelles with around 45–110 nm in size in aqueous solution spontaneously, which contained hydrophobic PCL core and hydrophilic PEEP shell. These star-shaped PCL-PEEP copolymers had low CMC values ranging from 5.74×10^{-4} to 3.87×10^{-3} g L^{-1}. The micelles can be used as carrier of hydrophobic paclitaxel with 1.62–3.46% (wt/wt) loading contents. Such colloid drug-delivery system with a low percentage of hydrophobic block length exhibited relatively rapid drug release. In addition, *in vitro* cytotoxicity assay of those copolymer micelles showed that these star-shaped copolymers are biocompatible. In conclusion, these biodegradable copolymers may be suitable for encapsulation and delivery of hydrophobic drugs.

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