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Amphiphilic Star-Shaped Poly(ε-caprolactone)-*block*-poly(L-Lysine) Copolymers with Porphyrin Core: Synthesis, Self-Assembly, and Cell Viability Assay

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ABSTRACT: Star-shaped copolymers poly(ε-caprolactone)-*bolck*-poly(ε-benzyloxycarbonyl-L-lysine) (SPPCL-*b*-PZLLs) with porphyrin core were synthesized by a sequential ring-opening polymerization (ROP) of CL and Nε-Benzyloxycarbonyl-L-lysine *N*-Carboxyanhydride. After the deprotection of benzyloxycarbonyl groups in polylysine blocks, the star-shaped amphiphilic copolymers SPPCL-*b*-PLLs were obtained. These amphiphilic copolymers can self-assemble into micelles or aggregates in aqueous solution. Investigation shows that the morphology of micelles/aggregates varied according to the change of pH values of media, indicating the pH-responsive property of SPPCL-*b*-PLL copolymers. Furthermore, associated with conjugated porphyrin cores, the SPPCL-*b*-PLL copolymers micelles showed a certain degree of Photodynamic Therapy (PDT) effects on tumor cells, suggesting its potential application as carrier for hydrophobic drug with additional therapeutic ability of inherent porphyrin segments. © 2013 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2014**, *131*, 40097.

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INTRODUCTION

Star-shaped polymers have attracted much attention due to the unique chemical and physical properties that are different from their linear counterparts.[1–10] Moreover, star-shaped amphiphilic copolymers can self-assemble into nanosized micelles in aqueous solutions.[11–16] Therefore, these copolymers have the potential applications in the biomedical fields, such as drug or gene delivery systems, if the components of the copolymers are biodegradable or biocompatible.[17–26]

Among the hydrophobic segments in the star-shaped copolymers, poly(ε-caprolactone) (PCL) has the potential application in biomedical fields due to its excellent properties.[27–36] PCL is one of the important aliphatic polyesters and has good biodegradability, biocompatibility, and drug permeability.[37–40] Moreover, PCL is hydrophobic polymer and can be used as the core of the self-assembled micelles. Wang et al. reported the synthesis and self-assembly of star amphiphilic PCL-*block*-poly(ethylene glycol) (PCL-*b*-PEG). Hydrophobic dyes and drugs can be encapsulated in the micelles.[41] Zhu et al. reported the preparation the drug-conjugated amphiphilic miktoarm star copolymers composed of 14 PCL arms and 7 PEG arms with β-cyclodextrin as core moiety via controlled ring-opening polymerization and click chemistry.[42] Qian et al. reported the self-assembled biodegradable micelles based on star-shaped PCL-*b*-PEG copolymers for chemotherapeutic drug delivery.[43]

In the star-shaped amphiphilic copolymers, poly(ethylene oxide) (PEO) is usually used as the hydrophilic segments due to the good hydrophilicty and biocompatiblility.[44–47] Moreover, in order to obtain the stimuli-responsive micelles, the environmental responsive polymeric segments were introduced to the star-shaped amphiphilic copolymers and used as the shell of the micelles. In our previous work, the temperature and (or) pH responsive star-shaped poly(L-alctide)-*block*-poly(2-(dimethylamino) ethyl methacrylate)) (PLLA-*b*-PDMAEMA) and PCL-*block*-poly(oligo(ethylene glycol) methacrylate) (PCL-*b*-POEGMA) copolymers and their micelles were prepared.[48,49]

Moreover, polypeptide hybrid copolymers consisting of synthetic polymers blocks and polypeptide segments represent a special type of the block copolymers. The presence of peptide segments endows block copolymers with intriguing supramolecular nanostructures through hierarchical self-assembly, partially due to the formation of characteristic protein folding motif via inter- and intra-

molecular interactions. Molecular structure of the polypeptide hybrid block can be tailor-made N-carboxyanhydride (NCA) ROP reaction technique. Many investigations have reported the self-assembly of peptide hybrid amphiphilic block copolymers consisting of hydrophobic synthetic block and hydrophilic polypeptides block in aqueous solution. Lubbert et al. reported the nonspherical assemblies generated from polystyrene-*block*-poly(L-lysine) (PS-*b*-PLL) polyelectrolyte block copolymers.[50] Deng et al. reported the preparation of PEG-*block*-PLLA-*block*-poly(L-glutamic acid) (PEG-*b*-PLLA-*b*-PLGA) triblock copolymer.[51]

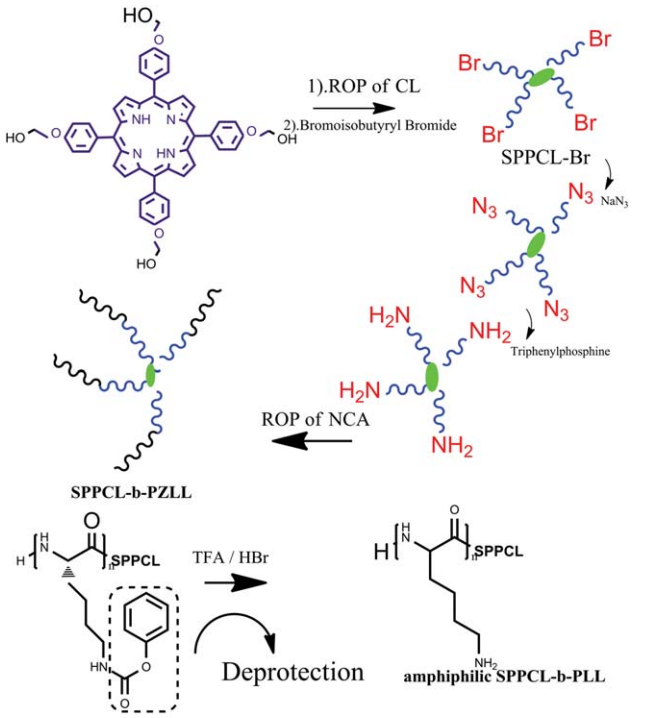
Porphyrin and porphyrin-like molecules hold promising functions for various applications containing light-harvesting materials, photosensitizers, and photodynamic therapy. Fréchet and Dong reported the preparation of star-shaped PCL by ROP with porphyrin core.[52,53] Holder and Cornelissen synthesized a series of star polymers with porphyrin core using ATRP.[54,55] These porphyrin-cored polymers cannot form micelles in water and therefore are limited in biomedical applications. Mineo et al. reported water soluble porphyrin with PEO arms.[56] However, most of the reported porphyrin-cored polymers did not present amphiphilic properties. Up to now, the self-assembly behavior of the star-shaped porphyrin-cored polymers containing biodegradable and biocompatible chains are rarely investigated and deserve further studied due to the potential applications in biomedical field.

In this paper, novel and well-defined star-shaped amphiphilic poly (ε-caprolactone)-*bolck*-poly(L-lysine) with porphyrin core (SPPCL-*b*-PLL) was synthesized by sequential ROP reactions. Namely, tetrahydroxyethyl-terminated porphyrin was sued as an imitator for the ROP of CL to prepare star-shaped poly(ε-caprolactone) with porphyrin core (SPPCL). Then, SPPCLBr macroinitiator was obtained by the reaction of SPPCL with 2-bromoisobutyryl bromide. After azide reaction with NaN_3, $SPPCLN_3$ was obtained. $SPPCLNH_2$ was prepared via the reaction of $SPPCLN_3$ with triphenylphosphine. SPPCL-*b*-PZLL was synthesized by ROP of Nε-Benzyloxycarbonyl-L-lysine *N*-Carboxyanhydride with $SPPCLNH_2$ macroinitiator. After the deprotection of benzyloxycarbonyl groups in polylysine blocks, the star-shaped amphiphilic copolymers SPPCL-*b*-PLLs were obtained (Scheme 1). In addition, the self-assembly behavior, pH-responsive property, and CMC of SPPCL-*b*-PLL were investigated with UV-visible spectrophotometer (UV-vis), transmission electron microscopy (TEM), and dynamic light scattering spectrophotometer (DLS). Finally, the cell viability assays of the SSPPCL-*b*-PLL micelles were investigated.

EXPERIMENTAL

Materials

Nε-Benzyloxycarbonyl-L-lysine (Nε-Z-Lys, >98%), triphosgene, sodium azide (NaN_3), hydrobromic acid in glacial acetic acid (33 wt %), triphenylphosphine (Aldrich), trifluoroacetic acid (TFA, >99%), tin 2-ethylhexanoate ($Sn(Oct)_2$) (Aldrich) and 2-bromoisobutyryl bromide (Aldrich) were used as received. THF, dimethylformamide, triethylamine, and dichloromethane were purified with CaH_2 by vacuum distillation. Star-shaped PCL with porphyrin core (SPPCL) was prepared according to our previous work.[49]



Scheme 1. Synthesis of amphiphilic star-shaped SPPCL-*b*-PLL copolymer. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Measurements

Attenuated total reflection fourier transform infrared (ATR FT-IR) spectra were conducted on an AVATAR 360 ESP FTIR spectrometer.

^{1}H-NMR were recorded on a 500 MHz DMX-500 Bruker Electrospin spectrometer with $CDCl_3$ or DMSO-d_6 as solvent at 25°C, using chemical shifts of tetramethsilane as $\delta = 0$ ppm for protons.

Gel permeation chromatography (GPC) measurement of polymer samples was performed using a Polymer Standards Systems apparatus with a Waters 150C separations module and a Waters differential refractometer. The molecular weight and molecular weight distributions were calibrated against polystyrene standards, with DMF as the eluent at a flow rate of 1 mL/min.

The hydrodynamic size and distribution of the copolymer micelles was determined by dynamic light scattering (DLS) using a Malvern Instruments Zetasizer Nano ZS with a He-Ne laser at 633. Polymeric micelles were prepared by membrane-dialysis, and then redispersed in deionized water at the concentration of 200 mg/L, sonicated for 30 s in an ice/water bath before filtered through a 450 nm filter.

The morphology of the micelles was determined by both transmission electron microscopy (TEM) and atom force microscopy (AFM). Samples for TEM images were taken on a Tecnai-12 Bio-Twin transmission electron microscope (FEI, Netherlands) operating at 120 kV, stained by 1% phosphotungstic acid aqueous solution. A small drop from the micelles solution (0.2 mg/mL, filtered through a 450 nm filter) was deposited onto carbon-coated copper TEM grid, after water evaporated under ambient atmosphere, depositing a small drop of 1% phospho wolframate aqueous solution to the copper grid. Wiping off

excess water with a filter paper, the grid was dried under ambient atmosphere at room temperature.

The atom force microscopy (AFM) images of the polymeric micelles were recorded on a SPA-300HV atomic force microscope (SII NanoTechnology, Japan). Using newly cleaved fresh mica surface as the substrate, a small drop of micelles solution was deposited on substrate without further staining, dried under ambient atmosphere to allow water tardily evaporated.

The critical micelle formation concentration (CMC) of SPPCl-*b*-PLL copolymer in aqueous solution was determined on LS55 luminescence spectrometer (Perkin-Elmer) with the aid of pyrene as a hydrophobic fluorescent probe. 0.2 mL of pyrene solutions (6×10^{-6} *M* in acetone) were added to containers and the acetone was allowed to evaporate. 2 mL of copolymer aqueous solutions with various polymer concentrations from 2×10^{-3} to 0.25 mg/mL were then added to the containers so that each container contained the same concentration (6×10^{-7} *M*) of excess pyrene residue but different polymer concentration. Excitation wavelength was carried out at 330 nm, and emission spectra ranging from 350 to 390 nm were recorded, while the bandwidths of both excitation and emission were 5 nm. The intensity I_{384} as a function of the polymer concentration was analyzed to measure CMC. This value was determined from the intersection of the tangent to the curve at the inflection with the horizontal tangent through the points at low concentration.

Preparation

Synthesis of Nε-Benzyloxycarbonyl-L-lysine N-Carboxyanhydride (Z-L-Lys NCA). The following reaction was carried out according to the literature.[57] In detail, *N*ε-benzyloxycarbonyl-L-lysine (4 g, 14.27 mmol) was dissolved in anhydrous THF (80 mL) and placed in a three-necked round-bottomed flask equipped with water condenser, funnel, and magnetic stirring bar. The mixture was left to reflux and kept in atmosphere by bubbling argon in the stirred reaction medium for 10 min. Triphosgene (1.49 g, 5.03 mmol) dissolved in anhydrous THF (25 mL), was added dropwise to the mixture under stirring over a period of 1 h. Then allow the reaction to proceed under argon flux until a clear solution was obtained. The mixture was filtered, concentrated, and precipitated in cold hexane twice, then dried under vacuum to yield (70%) a white solid.

Synthesis of Amino Terminated Star-Shaped PCL with Porphyrin Core (SPPCLNH$_2$). In order to obtain the amino terminated SPPCLNH$_2$, the hydroxyl groups of SPPCL were subsequently converted to bromine, azide, and amino groups by the reactions with 2-bromoisobutyryl bromide, sodium azide, and triphenylphosphine respectively. Typically, SPPCL (5 g, 0.289 mmol) was dissolved in anhydrous CH_2Cl_2 (100 mL) under stirring. To this solution was added triethylamine (351 mg, 3.47 mmol) under argon at 0°C with ice bath. Then 2-bromoisobutyryl bromide (798 mg, 3.47 mmol) in anhydrous CH_2Cl_2 (20 mL) was added dropwise to the mixture within 30 min. The reaction was stirred for 24 h at room temperature before the solution was washed with $NaHCO_3$ aqueous solution (100 mL), and deionized water (100 mL×2), then purified by precipitating from cold methanol. The resulting product SPPCLBr (3 g, 0.168 mmol) and sodium azide (0.873 g, 13.44 mmol) were dissolved in DMF (100 mL) under argon. The mixture was stirred at room temperature for 24 h. Then the SPPCLN$_3$ was obtained by precipitated in cold methanol, flittered, and dried till constant weight. The reduction of azide group into amino group was carried out according the literature.[58] In detail, SPPCLN$_3$ (2.58 g) and triphenylphosphine (0.20 g) were dissolved in DMF (30 mL). The solution was stirred for 2 h at room temperature before it was added 5 mL of deionized water. Allow the reaction to proceed for further 3 h at 90°C, and then the reaction mixture was cooled down to room temperature and precipitated out in acetone, filtered off, and dried under vacuum to yield (80%) a green solid.

Polymerization of Z-L-Lys NCA: Star-Shaped SPPCL-b-PZLL Copolymer With Porphyrin Core. The SPPCL-*b*-PZLL copolymer was synthesized from the Z-L-Lys NCA by using the amino-terminated macroinitiator SPPCLNH$_2$. A typical polymerization procedure was described as follows. The Z-L-Lys NCA (0.556 g, 1.816 mmol) was dissolved in anhydrous DMF. The calculated amount of initiator, SPPCLNH$_2$ (0.2 g, 0.0113 mmol) was added to the above solution. The reaction was allowed to proceed at 30°C for 50 h.

Deprotection of the Z Groups of SPPCL-b-PZLL Copolymer. The amine groups of SPPCL-*b*-PZLL were exposed by hydrolysis the benzyl carbamate (Z) group in TFA using 33 wt % hydrobromic acid solutions in glacial acetic acid. SPPCL-*b*-PZLL (500 mg) was dissolved in 5 mL of CF_3COOH, then excessive amount (2 times excess with respect to polypeptide) of HBr in HAc solution were added before the resulting solution was stirred under argon for 2 h at 0°C. The reaction solution was dialyzed against distilled water for 24 h with adjusting pH to 7 by adding ammonia water, and then the solution was dialyzed in distilled water for another 24 h. The green powder was gained after freeze drying the polymeric aqueous solution.

Preparation of SPPCL-b-PLL Micelles

Membrane-dialysis was used to prepare the SPPCL-*b*-PLL micelles. The freeze dried SPPCL-*b*-PLL (20 mg) was dissolved in 10 mL of DMF, and then the solution was transferred into a dialysis bag (MWCO: 8,000–12,000 Da) and dialyzed against distilled water, refreshed every 12 h for 72 h at room temperature to remove the DMF and to form the micelles.

RESULTS AND DISCUSSION

Synthesis of Four-Arm Star-Shaped Block Copolymer SPPCL-b-PLL With Porphyrin Core

The "grafting from" copolymerization of PZLL blocks was achieved by anionic ROP of N-ε-Z-Lys NCA, which mainly relied on the initiation of pre-modified functional amine groups in the end of star-shaped SPPCLNH$_2$ with porphyrin core. SPPCL-*b*-PZLL copolymers with different molecular weight were synthesized by varying the feed weight ratios of polypeptide monomer N-ε-Z-Lys NCA to the SPPCL macroinitiator. After hydrolyzing the benzyl carbamate (Z) group from PZLL blocks, two SPPCL-*b*-PLL copolymers with different length of hydrophilic PLL block were selected to assemble into micelles through dialysis against water. The above synthetic procedure can be mainly divided into two parts. One is the modification

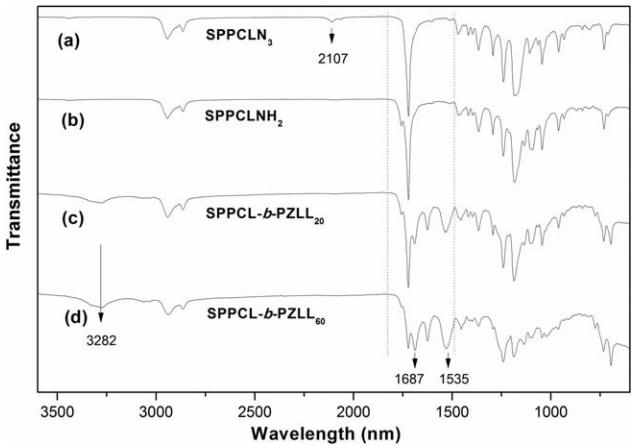


Figure 1. ATR FTIR spectra of (a) $SPPCLN_3$, (b) $SPPCLNH_2$, (c) SPPCL-*b*-PZLL, and (d) SPPCL-*b*-PLL.

of SPPCL, which includes the successive conversion of hydroxyl terminated group to bromide, then the conversion to azide group, finally to amine end-group on the PCL chain. In the other part, SPPCL-*b*-PLL was prepared by ROP of N-ε-Z-Lys NCA initiated by the primary amine on the end of modified PCL, and the hydrolysis of Z group in TFA/HBr. The ATR FT-IR spectra of initiator and SPPCL-*b*-PZLL are illustrated in Figure 1. A small peak appeared at 2107 cm^{-1} is ascribed to the stretching vibration of azide group. It is obvious that this peak completely disappeared after the reduction reaction by triphenylphosphine, which indicating complete conversion of primary amine from azide group. Meanwhile, it was also demonstrated by ^1H NMR spectrum as shown in Figure 2, results showing signal of the methyl protons close to nitrogen atom shifted to 1.47 ppm demonstrated that the azide groups were completely substituted. Primary amine group is expected to initiate the polymerization of peptide, in which the amino group of one amino acid combines with the carboxyl group of another. It is known that $SPPCLNH_2$ was used as initiator to afford the ROP of NCA to prepare PZLL blocks through a nucleophilic addition to the carbonyl group of the NCA. Therefore hydroxyl terminated SPPCL needs to convert to such a nucleophilic primary amide ended initiator, undergoing bromination, azide substitution, and reduction reactions in turn. As seen in our previous work,[49] with comparing the ratio of proton peaks at 4.01 ppm and 3.65 ppm and 9.16 ppm, which are the typical signals for $-CH_2-$ and $-CH_2OH$ protons on PCL moieties and the *β*-pyrrole on prophyrin moiety respectively, it is indicated PCL blocks on each arm of SPPCL contain 36 units of CL. After the bromination with hydroxyl end group, signal at 1.93, which disappeared after azide substitution, was corresponding to the methyl protons in the bromoethyl group ($-(CH_3)_2Br$). Therefore changes of signals in ^1H-NMR and ATR FTIR spectrum confirmed the successful synthesis of amide terminated $SPPCLNH_2$.

The structure of the SPPCL-*b*-PZLL copolymer was also revealed by ATR FTIR and ^1H-NMR. The ATR FTIR peaks at near 1687 and 1535 cm^{-1} are attributed to the characteristic bands of amide I and II bands of peptide bond unities. Besides, peak near 3282 cm^{-1} is caused by the stretching variation absorbance of N-H band. While feed weight ratios of Nε-Z-Lys NCA improved, these bands increased in intensity with respect to the typical absorption of carbonyl band in PCL block at 1722 cm^{-1}, which revealed the successful formation of peptide bonds and the combination of PCL and PZLL blocks initiated by primary amide contained. As seen in Figure 3, the proton signal at 4.99 and 7.32 ppm are attributed to the protons on

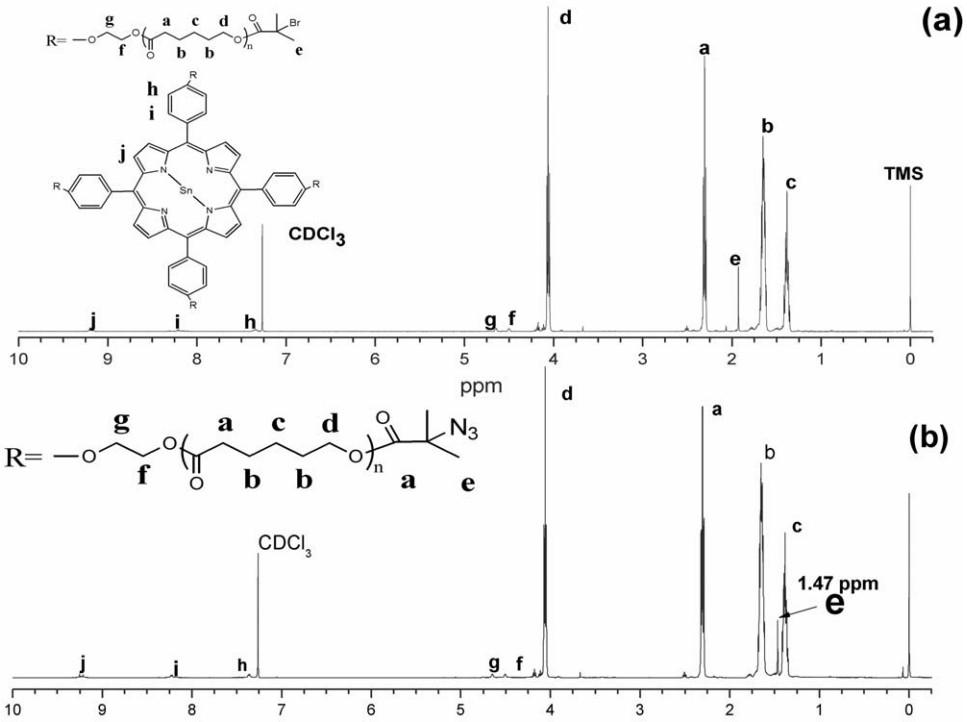


Figure 2. ^1H-NMR spectra of (a) SPPCLBr and (b) $SPPCLN_3$.

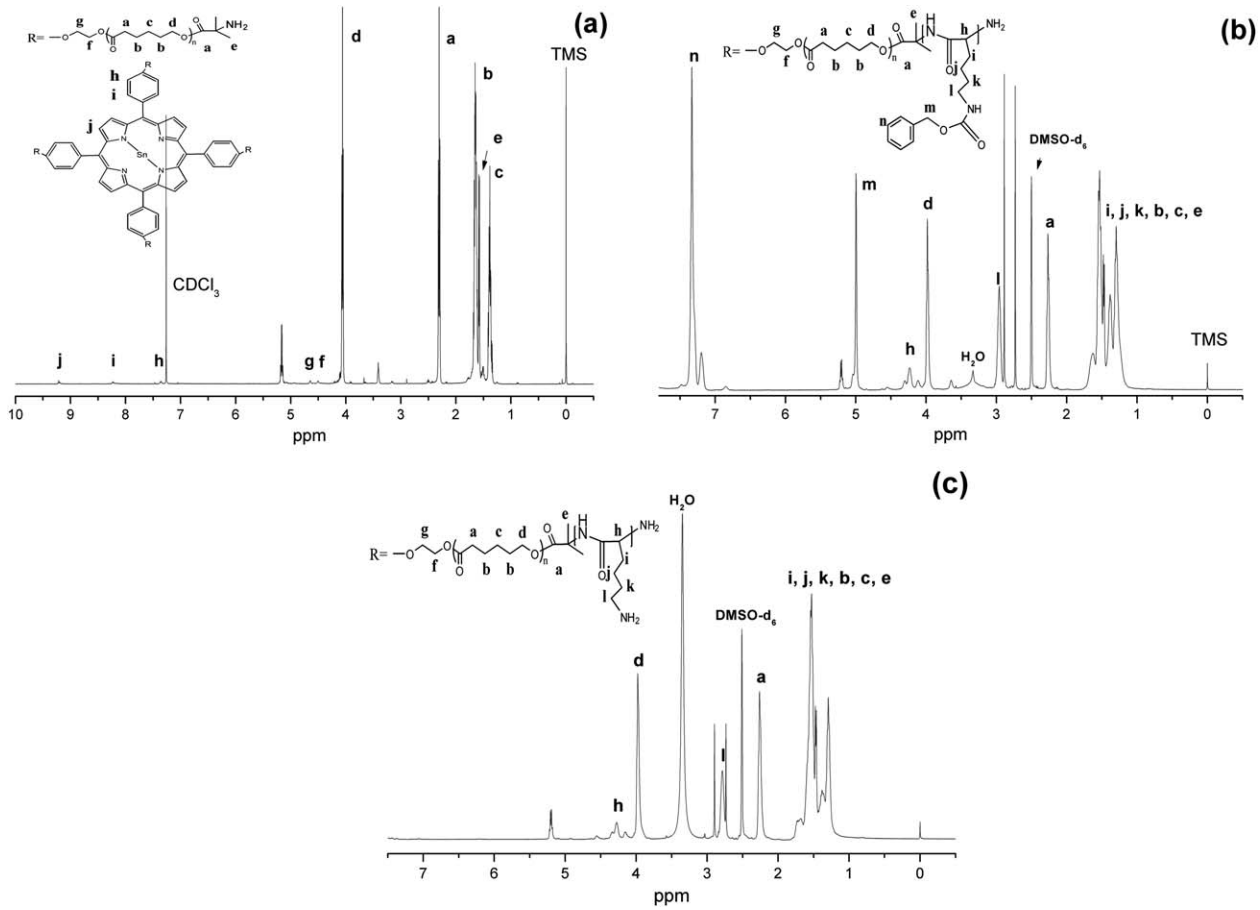


Figure 3. ^{1}H-NMR spectra of (a) SPPCLNH$_2$ ($CDCl_3$), (b) SPPCL-*b*-PZLL (DMSO-d_6), and (c) SPPCL-*b*-PLL (DMSO-d_6).

benzyl group and benzene ring of the protecting group respectively. Peaks at 1.29–1.53 ppm were assigned to methylene protons in amide contained side chain of the PZLL block, while the proton signals of methylene in side chain which are adjacent to the amide group appeared at 2.96 ppm. Signals for protons adjacent to tertiary carbon atom which consist in the backbone of polypeptide appeared at 4.22 ppm. Peaks at 1.38 ppm, 1.61 ppm, 2.26 ppm, and 4.01 ppm were assigned to protons of the PCL block. DP of PZLL block in the copolymer can be obtained from the integral ratio of $COCH_2$ in PCL to methylene protons in the side chain of PZLL at 2.96 ppm or the tertiary carbon protons in the backbone of PZLL at 4.22 ppm. By varying the feed proportion of N-ε-Z-Lys NCA, propagation of PZLL chain can be manipulated with the molar ratio of monomer to macro-initiator as shown in Table I and Figure 4. The number-average molecular weight of the resulting copolymers linearly increased, related to the molar ratio of NCA and macro-initiator SPPCLNH$_2$ when ROP of NCA was carried out for 50 h, which indicated that the primary amide terminated star-shaped SPPCLNH$_2$ could be used as effective propagation centers for a triple shield structure with an outer layer of PLL blocks as hydrophilic corona (after deprotection of Z group), and an inner layer of porphyrin centered PCL arms as hydrophobic segment. Moreover, the number-average molecular weight determined by NMR are closed to $M_{n,th}$, manifesting all of the four primary amide groups took part in ROP of NCA, showing reliability and effectiveness of the porphyrin-polyester molecule as macro-initiator. However, $M_{n,GPC}$ are lower than $M_{n,th}$, which is ascribed to the smaller hydrodynamic radius resulted by the star shaped structure. The benzyloxycarbonyl protective group of PZLL block was removed by acidolysis in TFA using a 33% solution of HBr in HAc. It can be seen in Figure 3(c), peaks at 7.33 and 5.99 ppm disappeared, suggesting that the deprotection of Z group was completed.

Table I. Results of the Polymerization of Amino Groups in SPPCLNH$_2$ with Various of N-ε-Z-Lys NCA Monomer[a]

Sample	[monomer]/[initiator]	$M_{n,NMR}$[b]	$M_{n,GPC}$[c]	M_w/M_n[c]
SPPCL$_{36}$-*b*-PLL$_{16}$	20	33,900	25,300	1.27
SPPCL$_{36}$-*b*-PLL$_{37}$	40	54,900	39,800	1.35
SPPCL$_{36}$-*b*-PLL$_{54}$	60	71,900	51,000	1.37
SPPCL$_{36}$-*b*-PLL$_{71}$	80	89,200	61,200	1.41

[a] Polymerization time = 50 h, polymerization temperature = 30°C.
[b] $M_{n,NMR}$ was determined by ^{1}H-NMR spectroscopy of SPPCL-*b*-PZLL copolymer.
[c] $M_{n,GPC}$ and M_w/M_n were determined by GPC analysis with polystyrene standards. THF was used as eluent.

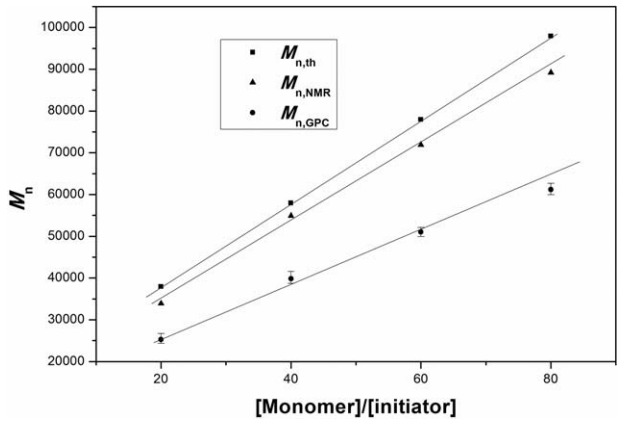


Figure 4. Dependence of M_n on the ratio of monomer to initiator [monomer]/[initator].

Self-Assembly Behavior and pH Responsiveness in Morphology of SPPCL-b-PLL

As an amphiphilic star-shaped copolymer, SPPCL-*b*-PLL can self-assemble into micelles. The hydrophobic chains of PCL bocks and their conjugated porphyrin segments are mainly in the core of the micelles, whereas hydrophilic arms of PLL are contributed to the outer corona, dispersing and stabilizing such core-shell structure in the aqueous solution. The critical micelles concentration (CMC), which is defined as the minor concentration of amphiphilic polymer to allow the formation of assembly in the aqueous solution, was obtained by steady-state fluorescent-probe method. Emission spectra of pyrene in the SPPCL-*b*-PLL aqueous solutions are shown in Figure 5. With increasing concentration of polymer solution, hydrophobic pyrene will preferentially transfer to the hydrophobic part; meanwhile repulsive interactions of aqueous phase allow hydrophobic of the amphiphilic stars to form stable cores of multi-molecular micelles in which pyrene could be concentrated. As a result, changes of molecular polar and increase of local concentration of pyrene would lead to its alteration of emission spectra and obvious enhancement of fluorescent intensity. As seen in Figure 5, obvious growing tendency in intensity can be found at relatively high concentrations after polymeric concentrations were increased above CMC, whereas spectra closet to each other at low concentrations. The ratio of intensity at 383 nm to 372 nm is plotted against the logarithm of polymer concentration, which showed an abrupt increase in magnitude at a certain concentration, indicating the assembly formation occurred at 27.9 mg·L^{-1} for SPPCL$_{36}$-*b*-PLL$_{37}$, and 11.2 mg·L^{-1} for SPPCL$_{36}$-*b*-PLL$_{71}$ respectively. It is noteworthy SPPCL$_{36}$-*b*-PLL$_{71}$ containing longer hydrophilic PLL blocks showed a relatively low CAC, which is ascribed the elongation of PLL blocks increased the number of C atoms in both backbone and side chains of PLL, resulting in the decrease of CMC. These low CMC provide a positive effect on stability of assemblies, allowing the applications in biomedical fields.

The morphology and size distribution of micelles were studied by transmission electron microscopy (TEM), atomic force microscopy (AFM), and dynamic light scattering

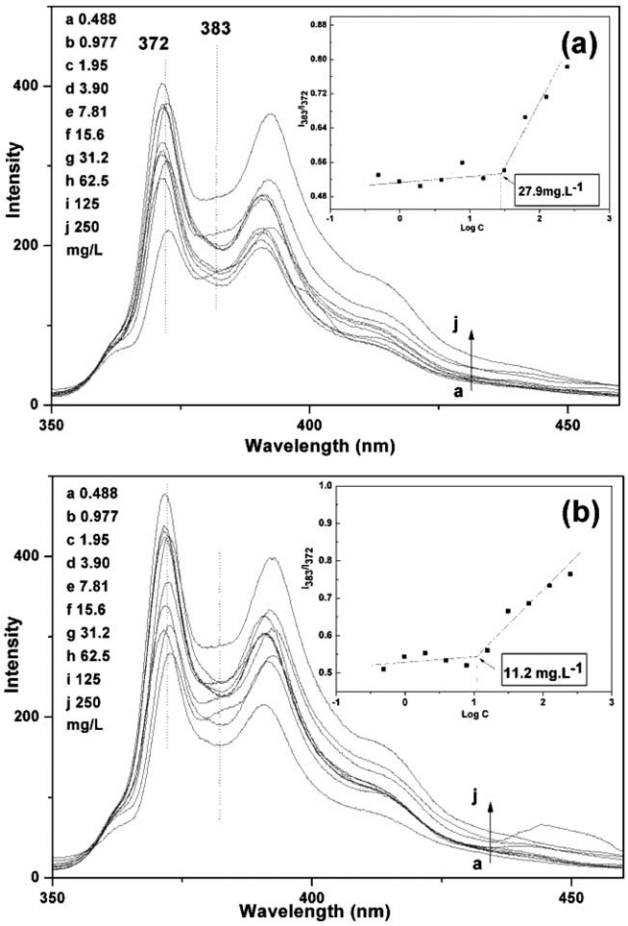


Figure 5. Critical micelles concentration (CMC) of (a) SPPCL$_{36}$-*b*-PLL$_{37}$ and (b) SPPCL$_{36}$-*b*-PLL$_{71}$ measured by steady-state fluorescent-probe method.

(DLS). After the deprotection of benzyl carbamate (Z) group, PLL blocks greatly obtained hydrophilicity from the exposed amine groups of the side chains, allowing the possibility of self-assembly due to an amphiphilic molecular structure. PLL blocks, as the outer hydrophilic layer, play an important role in stabilizing the aggregates. Their terminated amine groups on the side chains are inclined to be protonated, therefore enhancing the hydrophilicity. As shown in Figure 6, both SPPCL$_{36}$-*b*-PLL$_{37}$ and SPPCL$_{36}$-*b*-PLL$_{71}$ can form stable micelles in neutral water solution. Copolymer with shorter hydrophilic chains exhibited the formation of uniform spherical micelles, which have hydrophobic cores consisted of PCL-porphyrin segments and the hydrophilic shell in corona of the micelles. In contrast, morphology of micelles with longer hydrophilic chains became less uniform. In Figure 6(b), micelles tend to aggregate to larger ones. It is noteworthy that interface of smaller micelles existed obviously, despite that some aggregation had occurred. When pH was increased to 11, morphology of the micelles changed dramatically. In Figure 6(c) and (d), TEM morphology of SPPCL$_{36}$-*b*-PLL$_{37}$ and SPPCL$_{36}$-*b*-PLL$_{71}$ showed serious aggregation. Their size sharply increased and distribution of size became random and wide. Furthermore, copolymer

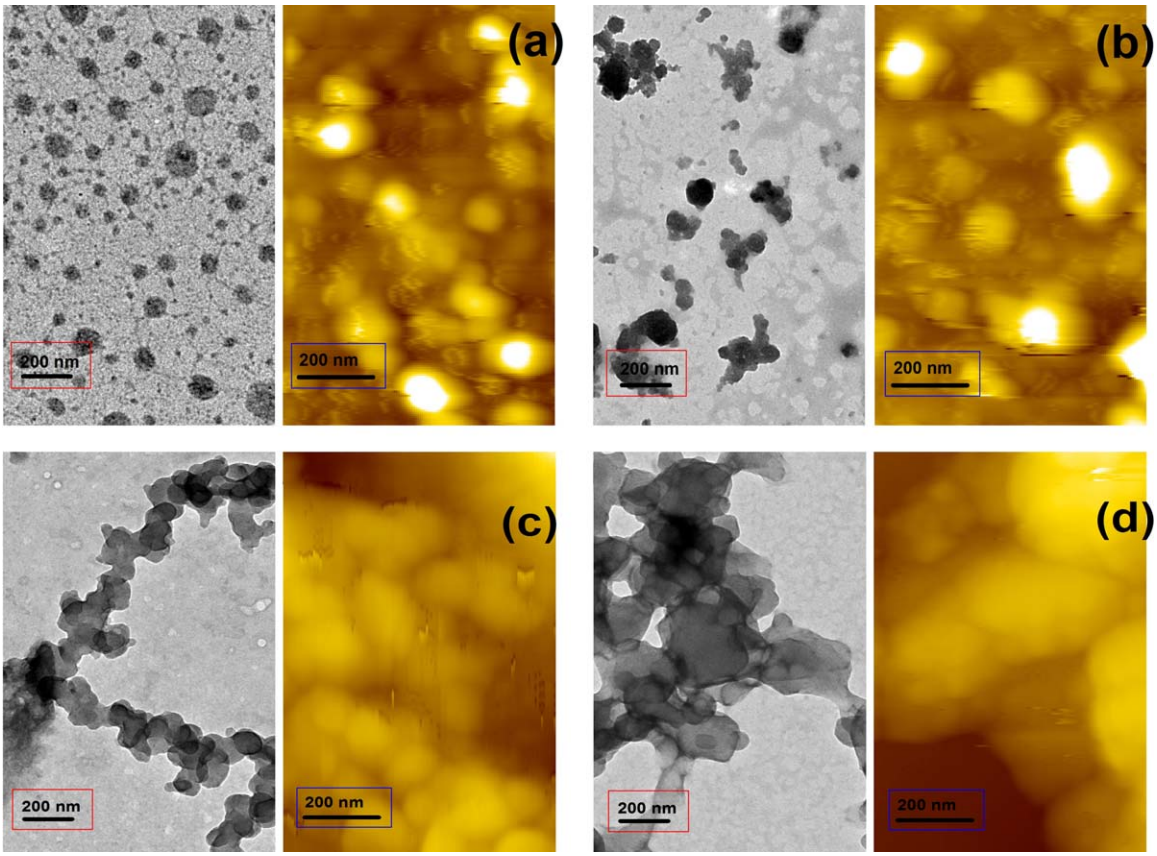


Figure 6. TEM and AFM images of (a) SPPCL$_{36}$-*b*-PLL$_{37}$ at pH 7, (b) SPPCL$_{36}$-*b*-PLL$_{71}$ at pH 7, (c) SPPCL$_{36}$-*b*-PLL$_{37}$ at pH 11, and (d) SPPCL$_{36}$-*b*-PLL$_{71}$ at pH 11 (concentration: 0.2 mg/mL). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

containing longer PLL blocks showed more tendency of aggregation, their self-assemblies inclined to form semi-interpenetrating structure.

As shown in Figure 7, the DLS results demonstrated the changes of morphology with TEM and AFM images. Size and size distribution of the micelles are showed in accordance with the samples of TEM and AFM. Obviously, the assemblies formed from longer block of PLL are more sensitive to pH changes, manifesting that PLL chains are the major factor contributing to the transformation in morphology. For example, comparing results between SPPCL$_{36}$-*b*-PLL$_{37}$ (a) and SPPCL$_{36}$-*b*-PLL$_{71}$ (b), whose length of PLL chains almost doubles has higher diameter on average. Their size distribution are relatively narrow and symmetrical to the normal distribution, demonstrating longer PLL block certainly has effect on the diameter of micelle, but not yet enough to render for serious aggregation taking place. When pH was switched to 11, size distributions became disorder and diameters were accessible to micron scale, according to DLS result. The copolymer bearing longer PLL blocks showed higher average diameter, which is accordance with the comparison at pH 7, suggesting PLL block is the critical part to effect the morphology of assemblies.

The pH responsive aggregation is attributed to the protonation equilibrium of PLL block. Apparently, under the pK_a 10.6 of PLL, the positively charged amide groups may enhance the interaction with water molecules, therefore increased water solubility well stabilizing the core-shell structure of formed micelles. When pH was increased above 10.6, the random coil conformation of the PLL corona of micelle may transform to alpha helix, in which intermolecular hydrogen bonding newly formed in the helical PLL chains caused the breakage of hydrogen bonding with water molecules, consequently may break down the equilibrium between the hydrophilic shell and hydrophobic core, aggregation among these unstable micelles occurred. As demonstrated in Figure 8, with increasing pH of water solution, size of micelles from the measurement of DLS showed an increased tendency.

Cell Viability Assays of SPPCL-b-PLL Micelles

Cell viability and cytotoxicity was performed in order to explore the potential therapeutic effect of the copolymer as its conjugated porphyrin is an important photosensitizer for Photodynamic Therapy (PDT). Human breast cancer MCF-7 cells were used to evaluate the cytotoxicity of the micelles self-assembled from copolymer SPPCL$_{36}$-*b*-PLL$_{71}$ and PCL-*b*-PLL block copolymer without porphyrin. The effect of the copolymer concentration on the proliferation of MCF-7 cells (for 24 h) was illustrated in Figure 9. As shown in Figure 9(a), the cell viability can keep high level at the concentration from 7.81 to 500 mg/mL for PCL-*b*-PLL micelle solution. However, as for SPPCL$_{36}$-*b*-PLL$_{71}$ micelle solution [Figure 9(b)], it can be seen that at low polymeric concentration,

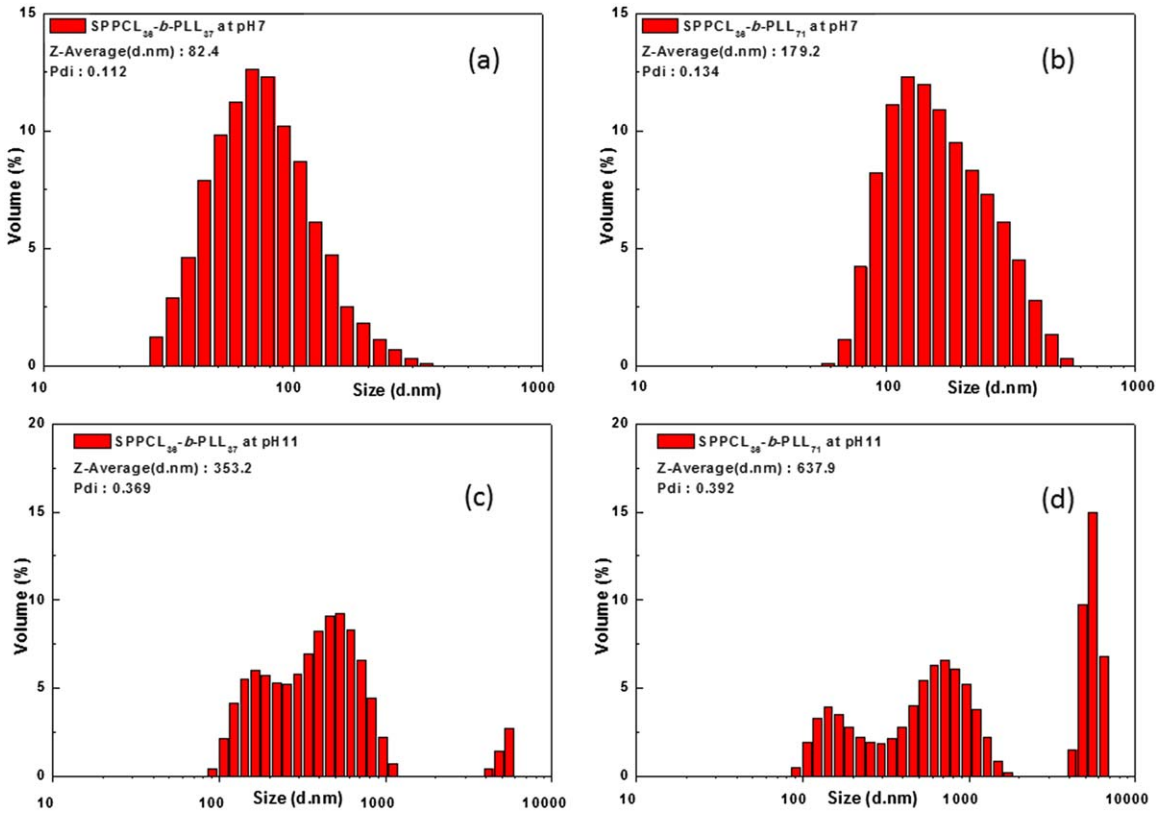


Figure 7. DLS results of the micelles of (a) $SPPCL_{36}$-*b*-PLL_{37} at pH 7, (b) $SPPCL_{36}$-*b*-PLL_{71} at pH 7, (c) $SPPCL_{36}$-*b*-PLL_{37} at pH 11, and (d) $SPPCL_{36}$-*b*-PLL_{71} at pH 11 (concentration: 0.2 mg/mL). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

micelles failed to obviously inhibit the cell proliferation. The cell viability kept relatively high when the polymeric concentration was below 30 $mg{\cdot}L^{-1}$. Reduction in viability apparently occurred as concentration increasing up to 62.5 $mg{\cdot}L^{-1}$, proliferation was inhibited by the conjugated porphyrin segment from the copolymer, representing that SPPCL-*b*-PLL micelles take on low cytotoxicity required as drug carriers, but also may provide the additional therapeutic function when used at high concentration.

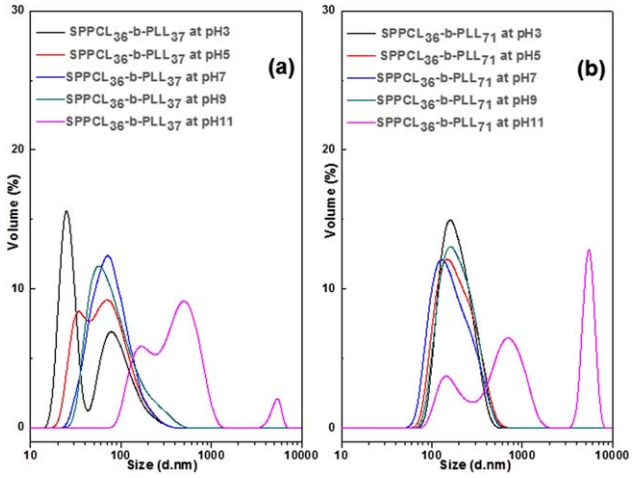


Figure 8. The change of the micelle sizes of (a) $SPPCL_{36}$-*b*-PLL_{37} and (b) $SPPCL_{36}$-*b*-PLL_{71} at various pH values. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

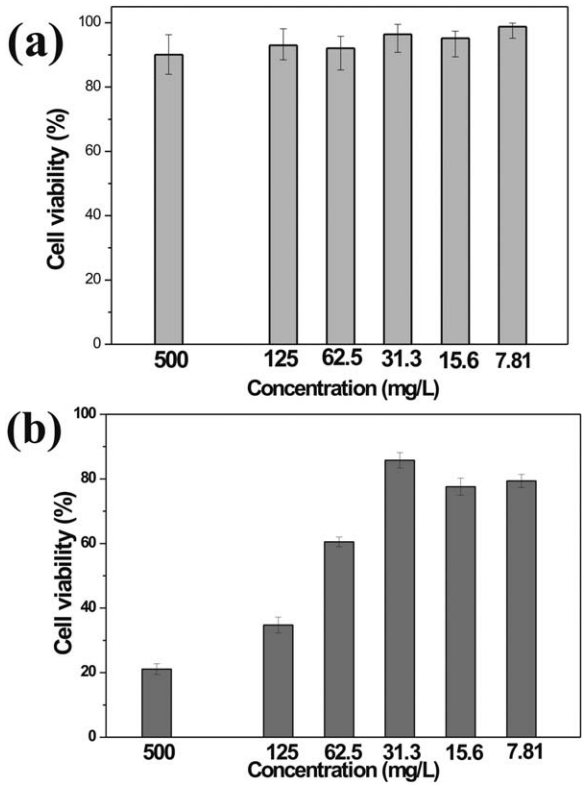


Figure 9. Cell viability assays of (a) PCL-*b*-PLL and (b) SSPCL-*b*-PLL micelles.

CONCLUSIONS

A degradable and pH responsive micellar aggregates were prepared from the porphyrin conjugated amphiphilic copolymer SPPCL-*b*-PLL, and synthesized via ROP of *N*-ε-Benzyloxycarbonyl-L-lysine *N*-Carboxyanhydride, which was initiated by amino terminated PCL with a porphyrin core. The morphology of micellar aggregates showed pH responsiveness. Furthermore, associated with conjugated porphyrin cores, self-assembled micelles showed a certain degree of PDT effects to tumor cells, suggesting its potential application as carrier for hydrophobic drug with additional therapeutic ability of inherent porphyrin segments.

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