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Synthesis, Self-Assembly, and Drug Release Behavior of Star-Shaped Poly(ε-caprolactone)-b-Poly(ethylene oxide) Block Copolymer with Porphyrin Core

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ABSTRACT: A serial of star-shaped poly(ε-caprolactone)-*b*-poly(ethylene oxide) (SPPCL-*b*-PEO) block copolymers with porphyrin core were successfully synthesized from ring-opening polymerization (ROP) of ε-caprolactone (CL) initiated with porphyrin core, followed by coupling reaction with a hydrophilic polymer poly(ethylene oxide) (PEO) shell. The structure of this novel copolymer were synthesized and thoroughly characterized by Nuclear Magnetic Resonance (NMR), Gel Permeation Chromatography (GPC), Fourier Transform Infrared Spectroscopy (FTIR). Notably, the as-prepared porphyrin-cored star-shaped copolymer could self-assembly into different structures determined by transmission electron microscopy (TEM) and dynamic lighting scattering (DLS), which provides the great potential of using this well-defined photodynamic therapy material for drug delivery system. Particularly, the doxorubicin-loaded SPPCL-*b*-PEO nanosphere exhibits property of pH-induced drug release. © 2014 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2014**, *131*, 40996.

KEYWORDS: biocompatibility; biodegradable; copolymers; drug delivery systems; morphology

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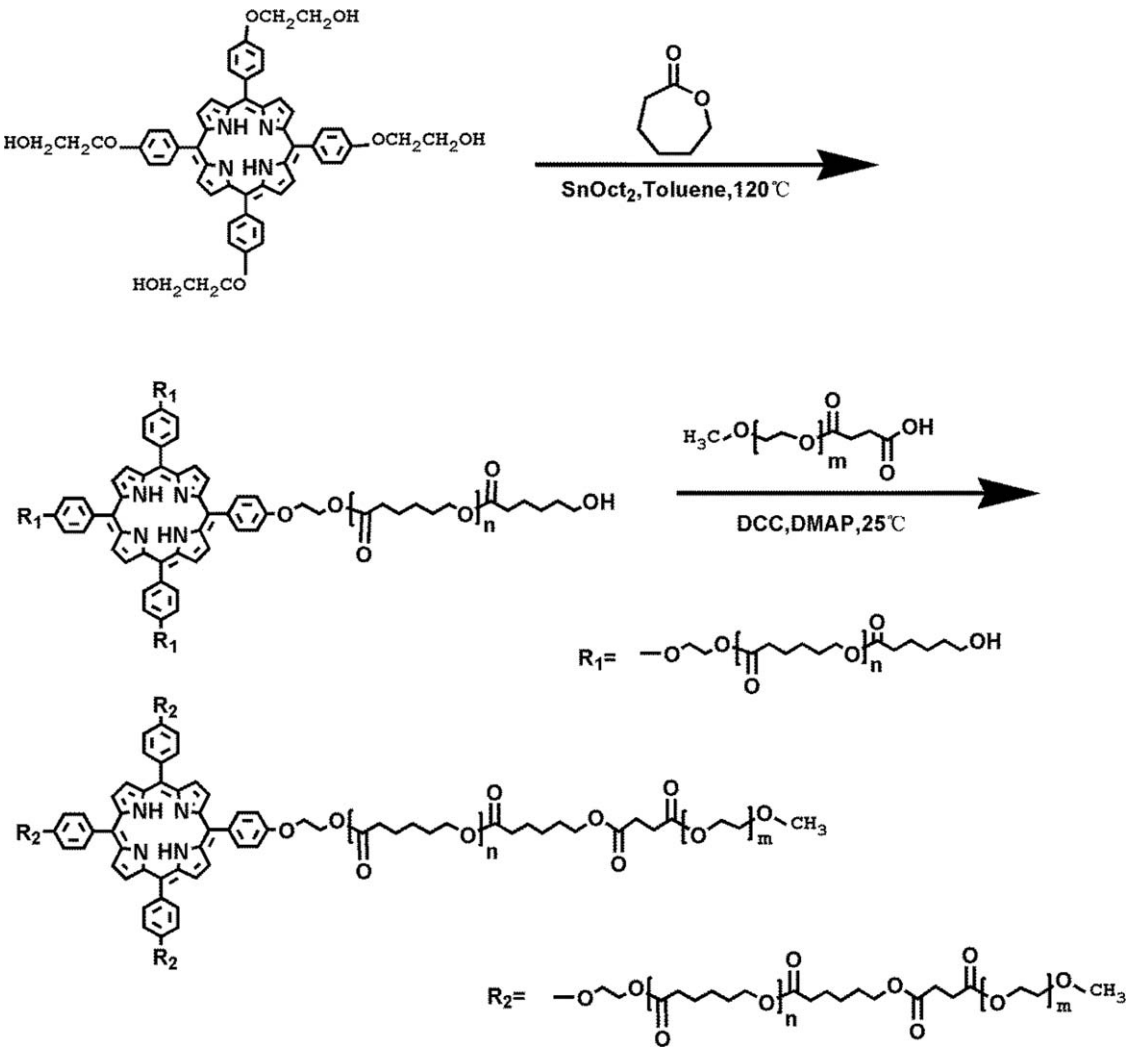
INRODUCTION

Porphyrin compounds for photodynamic therapy (PDT) is an emerging treatment material for eradicating premalignant and early-stage cancer, also reducing the tumor size in end-stage cancers by photosensitizers (PSs).[1–3] When the PSs are exposed to light of appropriate wavelength, they will produce highly reactive oxygen species (ROS) that induce an effective and selective destruction of diseased tissues without damage to the surrounding healthy tissues.[4,5] However, many drawbacks, such as molecular complexity, self-quenching, photo-toxicity to the skin induced by existing agents' hydrophilicity and hydrophobicity, greatly limit *in vivo* application.[6,7] Therefore, great efforts have been carried out in the delivery systems to address the problem of aggregation of porphyrin derivatives such as liposomes,[8] polymeric particles,[9] and hydrophilic polymer.[10]

Among the various vehicle used for delivery systems, star-shaped amphiphilic copolymers may be the best one since this material have well-defined and flexible architecture, controllable surface functionality, providing unique properties such as high surface reactivity, and low hydrodynamic radius.[11,12] Poly(ε-caprolactone) (PCL), as a U.S. Food and Drug Administration (FDA) approved biomedical polymers, have been increasingly studied as a biomedical and biocompatible material.[3,13,14] Recent years, Frechet,[15] Lai,[16] and our group[17,18]successfully synthesized a serial of porphyrin-cored PLA/PCL polymeric shell based on ring-opening polymerization (ROP). ROP is a form of chain-growth polymerization, in which the terminal end of a polymer acts as a reactive center, where further cyclic monomers join to form a larger polymer chain through ionic propagation. But these porphyrin-cored PLA/PCL polymers lack water solubility which greatly limited further application. Fortunately, these drawbacks might be tackled through the adjustment of polymer hydrophilicity-hydrophobicity balance by precisely control of branched macromolecular architecture. Presently, poly(ethylene oxide) (PEO) has been widely investigated



Scheme 1. Synthesis of SPPCL-b-PEO copolymers.

as an material in the last 2 decades, Using PEO with molecular weight less than 10,000, it is able to efficiently modulate the biodegradation and drug-release rates of the aforementioned PCL polymers. As an extension, Dong et al. used alkynyl-caped dendritic PCL and PEO-N_3 to obtained a serial of topological asymmetric linear/dendritic PCL-PEO by the method of click.[19] Quaglia et al. synthesize amphiphilic PCL-PEO triblock and star-shaped diblock copolymers and its ability to deliver drug was study.[20] Peng et al. used chlorin coupled with diblock copolymer PCL-PEO for dual chemo-photodynamic therapies.[10] REN et al. reported thermo sensitive porphyrin-cored amphiphilic PCL-*b*-POEGMA polymer.[21] But, to our knowledge, the report of star-shaped amphiphilic copolymers with porphyrin core is still limited. This work focusing on the prophyrin-cored star-shaped SPPCL-*b*-PEO copolymer not only improve the physical, biodegradation, biocompatibility properties of PCL-based biomaterials but also provide potentially copolymer for combined chemotherapy/PDT strategy in one delivery system.

Specifically, a novel and well-defined porphyrin-cored, star-shaped poly(ε-caprolactone)-*b*-Poly(ethylene oxide) (SPPCL-*b*-PEO) copolymer was synthesized. Namely, tetrahydroxyethyl terminated porphyrin was used as an initiator for the ROP of CL to prepare star-shaped poly(ε-caprolactone) with porphyrin core (SPPCL). Then, SPPCL-*b*-PEO was obtained by the reaction of SPPCL with monomethoxy poly(ethylene oxide) (CMPEO), as shown in Scheme 1. The molecular structure of the star-shaped prophyrin-cored SPPCL-*b*-PEO copolymer was thoroughly characterized by means of NMR, GPC, FTIR. Self-assembly behavior was studied by DLS and transmission electron microscope (TEM). Singlet oxygen ($^1O_2$) detection was investigated by fluorescent spectroscopy. In addition, its potential as a drug delivery carrier was also evaluated. Hydrophobic chemotherapeutic agent doxorubicin (DOX) as a kind of widely used anticancer drug can then be encapsulated into the core region of this micelle.

EXPERIMENTAL

Materials

4-dimethylamiopryidine(DMAP), dicyclohexylcarbodiimide (DCC), 1, 3-diphenylisobenzofuran (DPBF), doxorubicin (DOX) and pyrene were purchased from Aldrich used as received. Star-shaped porphyrin-cored poly(ε-caprolactone) (SPPCL) was synthesized from tetrahydroxyethyl terminated porphyrin, and ε-caprolactone (CL) according to the

Table I. Synthesis of SPPCL-*b*-PEO Copolymers with Porphyrin Core

Entry[a]	[M]/[I][b] (mol:mol)	$M_{n,GPC}$[c]	$M_{n,th}$[d]	$M_{n,NMR}$[e]	M_w/M_n[c]	f_{PCL}/f_{PEO}[f](%/%)	Yield (%)
$SPPCL_{15}$	60	14,243	6,327	7,703	1,31	100/0	91.8
$SPPCL_{24}$	120	21,133	14,552	11,356	1.43	100/0	96.3
$SPPCL_{15}$-PEO_{2000}	-	18,054	15,703	14,341	1.58	44.2/55.8	96.3
$SPPCL_{24}$-PEO_{2000}	-	21,314	19,809	17,543	1.56	54.4/45.6	84.0
$SPPCLl_{15}$-PEO_{500}	-	30446	27,703	24,758	1.32	19.2/80.8	73.3
$SPPCL_{24}$-PEO_{5000}	-	35,327	31,809	28903	1.44	30.8/69.2	75.2

[a] The subscript numbers represent the repeating units of polymers.
[b] M, ε-caprolactone monomer; I, initiator.
[c] M_w/M_n denotes the molecular weight distribution of polymer, where weight-average molecular weight (M_w) and number-average molecular weight (M_n) are determined by GPC in THF or/and DMF.
[d] $M_{n,th}$ denotes the theoretical number-average molecular weight, $M_{n,th} = [M]/[I] \times M_{monomer} \times$ yield $+ M_{initiator}$.
[e] $M_{n,NMR}$ was determined from the integral ratio of the signal from the ^{1}HNMR spectra.
[f] f_{PCL}/f_{PEO} denotes the weight fraction of PCL and/or PEO with in SPPCL-*b*-PEO copolymers, which was obtained from ^{1}HNMR.

literature procedure.[17] ^{1}HNMR ($CDCl_3$, TMS) of SPPCL sample: δ (ppm) = 1.35−1.45 (m, 192H, −$COCH_2CH_2C\mathbf{H}_2CH_2CH_2O$−), 1.55 − 1.79 (m, 384H, −$COCH_2C\mathbf{H}_2CH_2C\mathbf{H}_2CH_2O$−), 2.20 − 2.40 (m, 192H, −$COC\mathbf{H}_2CH_2CH_2CH_2CH_2O$−), 3.68 (t, 8H, −$CH_2OH$), 4.05 − 4.19 (m, 192H, −$COCH_2CH_2CH_2CH_2C\mathbf{H}_2O$−),4.48-4.53(t, 8H, $PhOCH_2\mathbf{CH}_2O$), 4.64-4.69(t, 8H, $PhO\mathbf{CH}_2CH_2O$),9.16(s, 8H, β-pyrrole-H), 8.18(d, 8H, m-Ar-H), 7.35(d, 8H,o-Ar-H). Carboxyl-Terminated poly(ethylene oxide) (CMPEO) was synthesized from poly(ethylene oxide) methyl ether and succinic anhydride according to the literature procedure (90.0% yield).[11] [11]Poly(ethylene oxide) methyl ether purchased from Aldrich ($M_n \sim 2000$, $M_w/M_n = 1.06$; $M_n \sim 5000$, $M_w/M_n = 1.04$) was dried at 50°C *in vacuo* overnight, and its purity was 100% within the error of ^{1}HNMR measurement. ^{1}HNMR ($CDCl_3$, ppm): 3.56–3.75 (—O—CH_2-CH_2-O—), 3.40 (CH_3-O—), 4.28 (—CH_2-OC—O—), 2.67 (—O—OC—CH_2-CH_2-CO—O—). Dimethyl formamide (DMF) and dichloromethane (CH_2Cl_2) and toluene were dried and distilled from CaH_2 and stored under a dry nitrogen atmosphere. All other reagents and solvents were used without further purification.

Synthesis of the Star-Shaped Amphiphilic Porphyrin-Cored Copolymers SPPCL-*b*-PEO

A typical polymerization was carried out as follows: $SPPCL_{24}$ (M_n = 11,356, 113.6 mg, 0.01 mmol), $CMPEO_{2000}$ (M_n = 2000, 130.7 mg, 0.0065 mmol), DCC (16.3 mg, 0.08 mmol), and DMAP (1.9 mg, 0.015 mmol) were put into the tube and then added 4 mL of anhydrous CH_2Cl_2. The mixture solution was stirred for 24 h at room temperature under a nitrogen atmosphere. The resulting mixture was filtered to remove by-product dicyclohexylcarbodiurea (DCU) and precipitated into diethyl ether anhydrous. The purified SPPCL-*b*-PEO was purified by solvent extraction with diethyl ether/benzene (2:1 v/v) as a cosolvent, and then methanol (50.0 mL) was used to completely extract unreacted CMPEO. The purple powder was dried overnight *in vacuo* at 40°C to give 126.2 mg $SSPCL_{24}$-*b*-PEO (65.1% yield).

Preparation of Aggregates in Water[22]

SPPCL-*b*-PEO copolymer was dissolved in DMF (1 mg/mL), followed by added distilled water dropwise using a microsyringe. After stirring for 24 h dialyzing against distilled water was used for 3 days to guarantee DMF was completely removed. The morphology of aggregates was determined by TEM.

Measurement of Critical Micelle Concentration

According to the literature,[23] critical micelle concentration (CMC) of SPPCL-*b*-PEO copolymer in aqueous solution was studied with pyrene as a hydrophobic fluorescent probe. Aliquots of pyrene solutions ($6 \times 10^{-6} M$ in acetone, 10 μL) was added to containers, then the acetone was allowed to evaporate. One milliliter of copolymer aqueous solution with a particular concentration was added to the container, which contained the pyrene residue. Each aqueous sample solutions with various polymer concentrations from 5×10^{-5} to 1.0 mg/mL and containing pyrene residue at the same concentration of 6×10^{-7}mol/L. Emission wavelength was carried out at 668 nm, and excitation spectra were recorded ranging from 300 to 360 nm. Both excitation and emission bandwidths were 5 nm. From the pyrene excitation spectra, the intensity ratio I_{334} was analyzed as a function of the polymer concentration. A CMC was determined from the intersection of the tangent to the

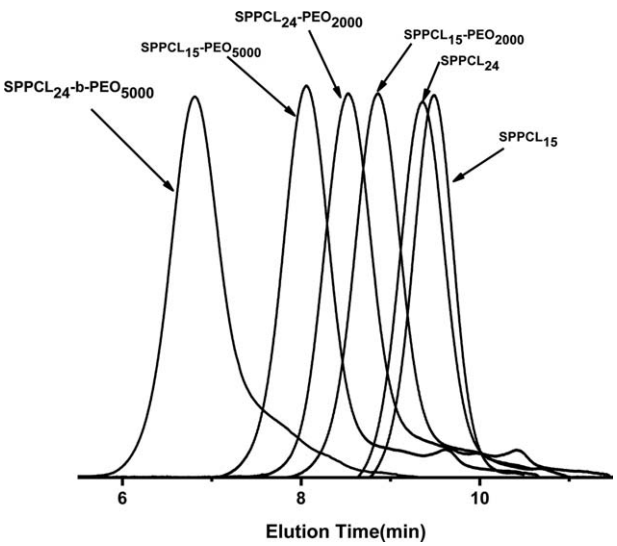


Figure 1. GPC traces of the SPPCL and the SPPCL-*b*-PEO samples.

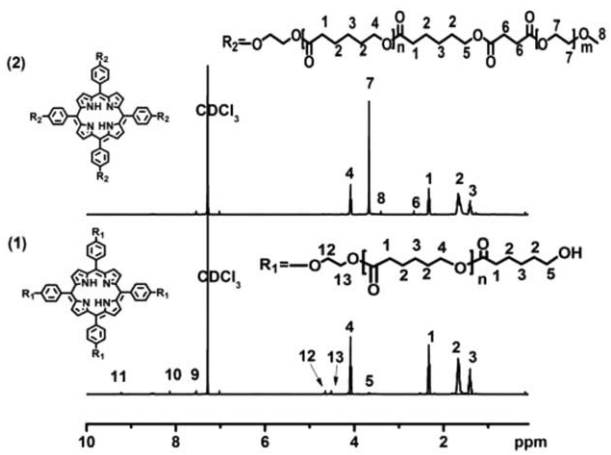


Figure 2. ^{1}HNMR spectra of SPPCL (1) and SPPCL-*b*-PEO (2) in $CDCl_3$.

curve at the inflection with the horizontal tangent through the points at low concentration.

Detecting Singlet Oxygen ($^{1}O_2$) Production

In our study, chemical method was proposed for detecting $^{1}O_2$ generated by SPPCL-*b*-PEO photosensitizers.[21] In this study, we used 1, 3-diphenylisobenzofuran (DPBF) as a probe to detect the singlet oxygen. DPBF reacts irreversibly with $^{1}O_2$ that causes a decrease in the intensity of the DPBF absorption band at 456 nm. In a typical experiment, $SPPCL_{24}$ and $SPPCL_{24}$-*b*-PEO_{5000} were respectively dissolved into DMF at concentration of 1.5×10^{-4} mol/L, and then these sample solutions (50 μL) were added 5 mL of DPBF in DMF (10^{-6} mol/L). The solutions were irradiated with a 650 nm laser source (5 mw) and their absorbencies at 456 nm were recorded every 30 s in a luminescence spectrometer.

Preparation of DOX-Loaded Nanoparticles in Aqueous Solution

By means of a dialysis technique,[24] SPPCL-*b*-PEO_{5000} (5 mg) and DOX hydrochloride (1 mg, 1.7 μmoL) were dissolved in 6.5 mL of DMF, in which 2.6 μmoL triethyl amine (Et_3N) (2.6 μmoL) was added to neutralize HCl in solution. Totally, 5 mL

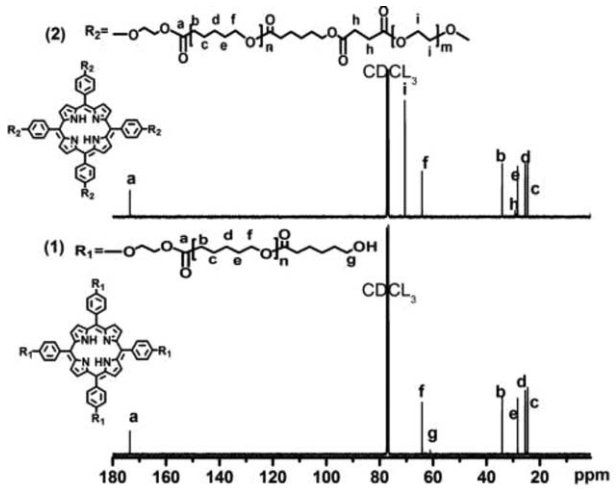


Figure 3. ^{13}CNMR spectra of SPPCL and SPPCL-*b*-PEO.

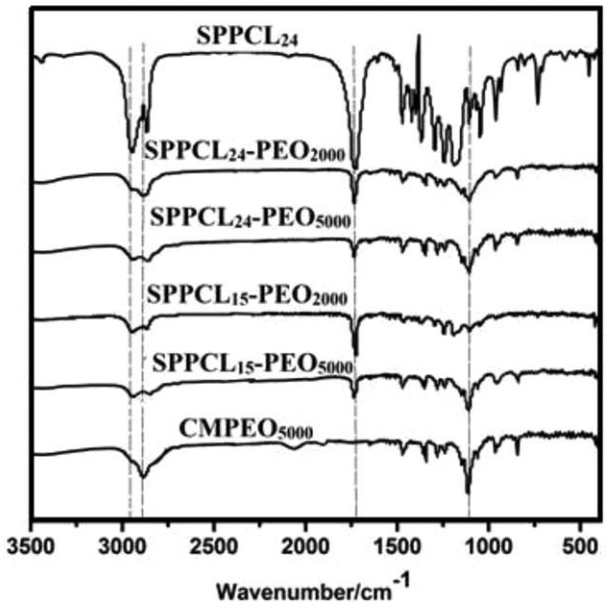


Figure 4. FTIR spectra of SPPCL, CMPEO, and SPPCL-*b*-PEO.

distilled water was injected by a microsyringe to form nanoparticles. The obtained nanoparticles solution was then added into a dialyzing membrane and subjected to dialysis against distilled water for 24 h. Then, the DOX-loaded nanoparticles solution was lyophilized and stored at 4°C. The DOX-loaded nanoparticles (1 mg) was dissolved in 5 mL of DMF and The loading efficiency of DOX in SPPCL-*b*-PEO was determined at an absorbance of 500 nm by UV-vis. Loading efficiency (L.E.) (%) = (weight of DOX in the SPPCL-*b*-PEO/weight of the feeding DOX) × 100%. The calibration curve of DOX in aqueous solution is y (abs) = 0.00116 + 19.5888× (C: mg/mL).

In Vitro DOX Release Studies of DOX-Loaded Nanoparticles

The lyophilized DOX-loaded nanoparticles (1.5 mg) were added into 1 mL of buffer solution (pH = 7.4 or 5.5) followed by put into a dialyzing membrane. The dialyzing membrane was put into 30 mL of buffer solution container at 37°C. The

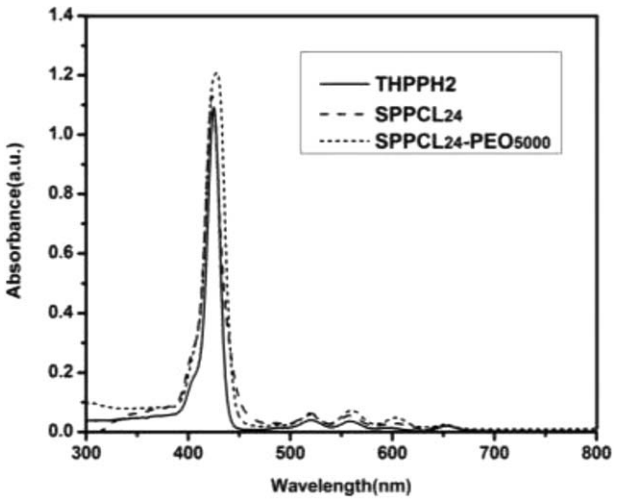


Figure 5. UV-vis spectra of HTPPH2, SPPCL, and SPPCL-*b*-PEO in DMSO solution.

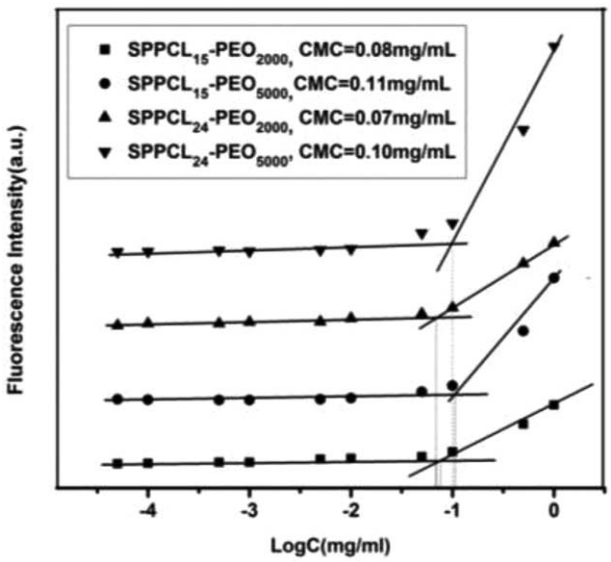


Figure 6. Fluorescent spectra and CMC trace of SPPCL-*b*-PEO.

DOX-released solution was extracted 3 mL periodically (3, 6, 9, 21, 33, 45, 57, 81, 105 h) followed by added 3 mL buffer solution, and by using UV–vis at 500 nm, the quantity of DOX released from nanoparticles was measured at room temperature.

Nuclear Magnetic Resonance Spectroscopy (NMR)

^{1}HNMR and ^{13}CNMR spectroscopy was performed on a Varian Mercury-400 spectrometer. Tetramethylsilane was used as an internal standard.

Gel Permeation Chromatography

Molecular weights and molecular weight distributions of the polymers were determined on GPC (Perkin-Elmer Series 200) and a refractive index detector at 30°C, The elution phase was DMF (0.01 mol/L LiBr) (elution rate: 1.0 mL/min), and polystyrene used as the calibration standard.

Fourier Transform Infrared

Fourier transform infrared (FTIR) spectra were recorded on a Nicolet FTIR spectrophotometer (Nexus 470, Thermo Electron Corporation) at frequencies ranging from 400 to 4000 cm^{-1}. Samples were thoroughly mixed with KBr and pressed into pellet form.

Fluorescence Spectra Analysis

Fluorescence spectra were performed at room temperature using a luminescence spectrometer (CaryEclipse, AUS).

Dynamic Light Scattering Analysis

The mean size of nanoparticles was determined by DLS using a Malvern Nano_S instrument (Malvern, UK). The solution of nanoparticles was performed at a scattering angle of 90°C and at 25°C. All the measurements were repeated three times, and the average values reported were the mean diameter ± standard deviation.

Transmission Electron Microscope

TEM micrographs were taken with a JEOL-JEM-2010 (JEOL, Japan) operated at 200 KV. One drop of aggregates solution was deposited onto the surface of 300 mesh Formvar-carbon film-coated copper grids. Excess solution was quickly wicked away

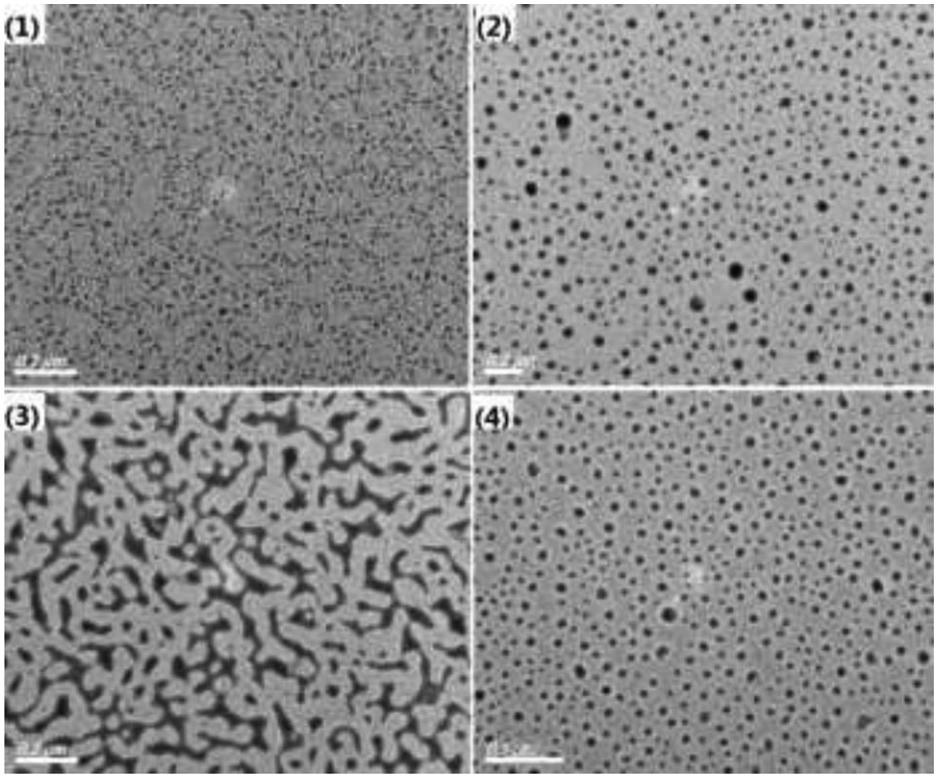


Figure 7. TEM photographs and nanoparticles size distribution of amphiphilic star-shaped SPPCL-*b*-PEO block copolymers: $SPPCL_{15}$-*b*-PEO_{2000} (1), $SPPCL_{15}$-*b*-PEO_{5000} (2), $SPPCL_{24}$-*b*-PEO_{2000} (3), and $SPPCL_{24}$-*b*-PEO_{5000} (4).

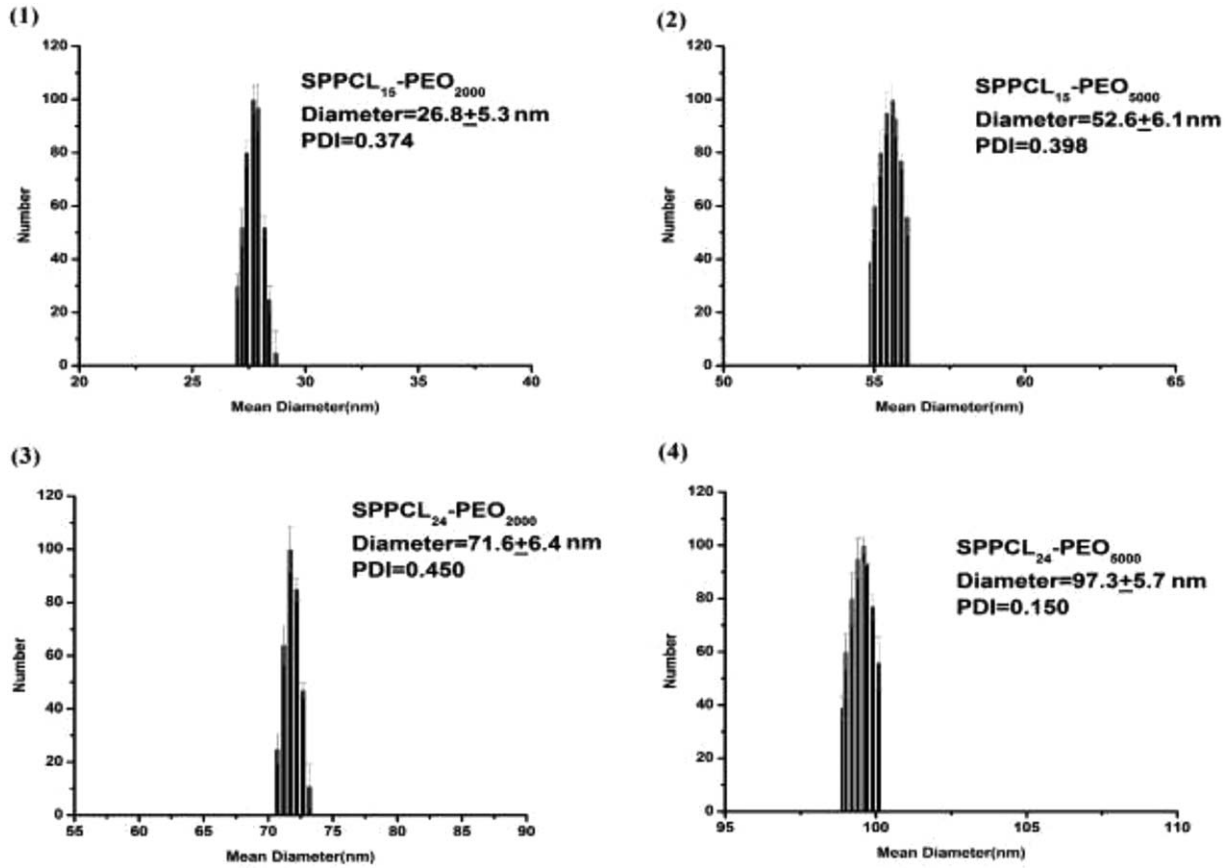


Figure 8. Nanoparticles size distribution of the SPPCL-*b*-PEO block copolymers in aqueous solution at room temperature: $SPPCL_{15}$-*b*-PEO_{2000} (1), $SPPCL_{15}$-*b*-PEO_{5000} (2), $SPPCL_{24}$-*b*-PEO_{2000} (3), and $SPPCL_{24}$-*b*-PEO_{5000} (4).

with a filter paper. The image contrast was enhanced by negative staining with phosphotungstic acid (0.5 wt %).

RESULTS AND DISCUSSION

Synthesis of Star-Shaped Amphiphilic Porphyrin-Cored Copolymers SPPCL-*b*-PEO

In our study, using the SPPCL polymers reacted with CMPEO and a serials of star-shaped amphiphilic porphrin-cored $SPPCL_{15}$-PEO_{2000}, $SPPCL_{15}$-PEO_{5000} $SPPCL_{24}$-PEO_{2000}, and $SPPCL_{24}$-PEO_{5000} were obtained, which have been summarized in Table I. Notably, the number–average molecular weight of the as synthesized polymers SPPCL-*b*-PEO determined by GPC ($M_{n,\mathrm{GPC}}$) were in well control when reacted with different molecular weight of CMPEO (Figure 1). Moreover, the polymer molecular weight determined by ^{1}HNMR ($M_{n,\mathrm{NMR}}$) is reasonably consistent with the theoretical molecular weight of polymer ($M_{n,\mathrm{th}}$), where $M_{n,\mathrm{th}} = [M]/[I] \times M_{\mathrm{monomer}} \times \mathrm{yield} + M_{\mathrm{initiator}}$. Furthermore, both ^{1}HNMR and ^{13}CNMR of the purified block copolymers confirmed that each arm of the SPPCL precursor was successfully coupled with CMPEO. The ^{1}HNMR spectrum of SPPCL-*b*-PEO polymer is shown (Figure 2). The characteristic signals of PCL(1.35−1.45 (δH^3), 1.55−1.79 (δH^2), 2.20−2.40 (δH^1), and 4.05−4.19 ppm (δH^4), PEO(3.375 ppm (δH^8), 3.637 ppm(δH^7),) and the porphyrin core initiator (4.48 ppm (δH^{13}), 4.64 ppm(δH^{12}), 7.52 ppm(δH^9), 8.11 ppm(δH^{10}), and 9.21ppm(δH^{11})) were observed. Moreover, ^{13}CNMR spectra represented that the end-group peaks of the SPPCL precursor at 61.3 ppm (δH^g) completely disappeared in the related SPPCL-*b*-PEO copolymer, and this phenomenon confirmed that all the hydroxyl groups in SPPCL were completely reacted with CMPEO within the limit of ^{13}CNMR measurements (Figure 3).

The FTIR results of SPPCL and SPPCL-PEO copolymers were shown in Figure 4. The distinct stretching bands at 2948 cm^{-1} (CH), 1725 cm^{-1} (C═O) and 730 cm^{-1} for PCL block, and the intense stretching bands at 2888 cm^{-1} and 843 cm^{-1} were assigned to the PEO block (Figure 4). Meanwhile, it is also observed that the relative intensity of aliphatic CH stretching band of PCL at 2948 cm^{-1} decreased while that of CH band of PEO at 2888 cm^{-1} increased. In all, the above results indicate that well-defined star-shaped porphyrin-cored SPPCL-*b*-PEO copolymers with different compositions were successfully synthesized.

UV-vis Analyses

The obtained SPPCL-*b*-PEO copolymers were further characterized using UV-vis spectroscopy (Figure 5). The UV-vis spectra of both SPPCL-*b*-PEO and porphyrin showed the Soret (435 nm) and Q bands (500–700 nm) which are known to be the characteristic of porphyrin. This proves that porphyrin moiety still retained the luminescent property within SPPCL-*b*-PEO. In other words, it means that the porphyrin could keep its luminescent feature which is important for cancer treatment.

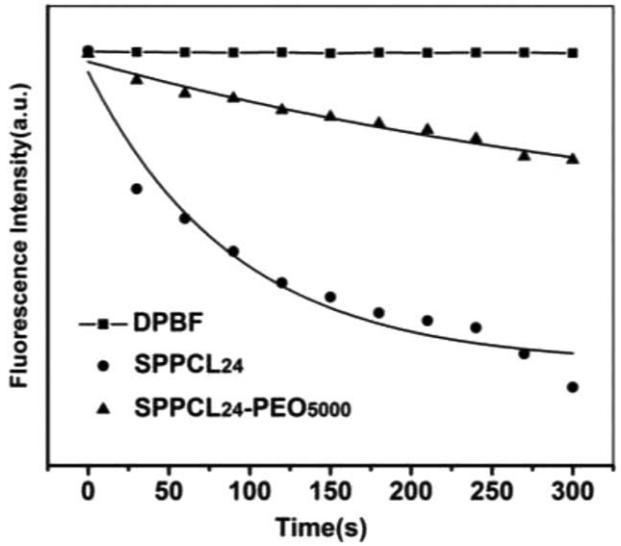


Figure 9. Fluorescence intensity (at 456 nm) decay curves of DPBF with SPPLA-*b*-PEG or SPPCL as function of time with a laser source (650 ± 10 nm, 5 M_w).

Thus, this will potentially enable SPPCL-*b*-PEO for the biological probe and PDT applications.[25,26]

Self-Assembly Properties of SPPCL-*b*-PEO Copolymers

It is well known that CMC was an important parameter for the thermodynamic stability of self-assembled aggregate in aqueous solution. Thus we examined the CMC values of these star-shaped porphyrin-cored SPPCL-*b*-PEO copolymers by the fluorescence technique using pyrene as a probe. In the emission spectra, fluorescence intensity increases with increase in concentration of copolymer. This was because pyrene preferentially aggregates into the hydrophobic micelle cores with a change of the photophysical properties. From the plot of fluorescence intensity versus polymer concentration, fluorescence intensity is essentially constant at low concentration. Above that concentration, the fluorescence intensity increased substantially, indicating the incorporation of pyrene in the hydrophobic region of aggregates. The CMC values were 0.08, 0.11, 0.07, and 0.10 mg/L for $SPPCL_{15}$-*b*-PEO_{2000}, $SPPCL_{15}$-*b*-PEO_{5000}, $SPPCL_{24}$-*b*-PEO_{2000}, and $SPPCL_{24}$-*b*-PEO_{5000}, respectively (Figure 6). It was noticed CMC values that increased with increasing block length of PEO, which was in a good agreement with that reported in other amphiphilic copolymers.[27,28]

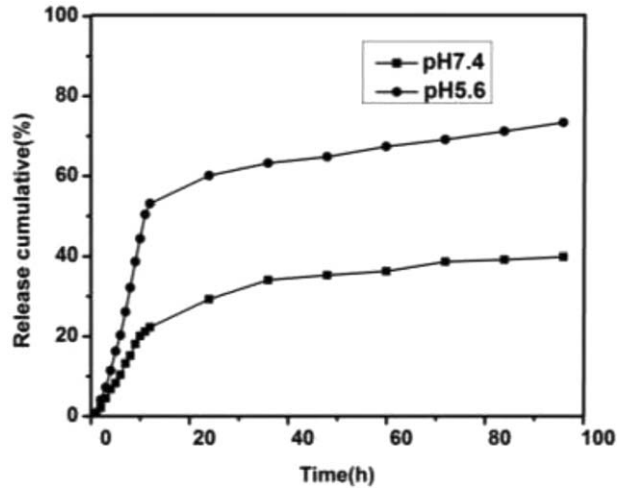


Figure 10. Doxorubicin release curve of $SPPCL_{24}$-PEO_{5000} nanospheres in PBS solutions of pH7.4 and 5.6 at 37°C.

Both the morphology and the average size of the self-assembled aggregates from these copolymers were investigated by TEM and DLS (Figures 7 and 8). To investigate the effect of hydrophilic PEO block length and/or the weight fraction (f_{PEO}) on morphology of the nanoparticles, the hydrophobic PCL block was kept equal to 24 or 15 repeating units. When the PEO block was short, (e.g., $SPPCL_{24}$-*b*-PEO_{2000}, f_{PEO} = 45.6%, 71.6 ± 6.4nm; $SPPCL_{15}$-PEO_{2000}, f_{PEO} = 55.8%, 26.8 ± 5.34 nm), the worm-like aggregates were obtained, As the weight fraction of PEO block increased, the diameter of normally spherical micelles were presented for $SPPCL_{15}$-EO_{5000} (f_{PEO} = 80.8%, 52.6 ± 6.15 nm) and $SPPCL_{24}$-*b*-PEO_{5000} (f_{PEO} = 69.2%, 97.3 ± 5.72nm) sample. These micellar aggregates were bigger than the conventional polymeric micelles usually with a diameter of 10–50 nm, which suggests that they should not have the simple core/shell micells structure formed from the conventional amphiphilic block copolymers. These bigger aggregates showed that they probably formed from the intermicellar aggregation of simple core-shell micelles (core, hydrophobic SPPCL; shell, water-soluble glycopolymer), which is induced by the strong hydrogen-bond interactions among the shell.[12] These results indicate that nanoparticles with micelles and worm-like morphologies could be fabricated by adjusting the weight fraction of PEO block within these star copolymers.[17]

Singlet Oxygen ($^1O_2$) Production of SPPCL-*b*-PEO Plus Irradiation

$^1O_2$ is an excitation state of oxygen molecule which has played a unique role in PDT. The generation of singlet oxygen by photosensitizer was detected chemically using the DPBF as a detector. Figure 9 shows the decrease in fluorescence intensity at 456 nm as a function of irradiation time. As shown in Figure 9, both SPPCL and SPPCL-*b*-PEO confirmed the $^1O_2$ generation from copolymer. However, when compared with SPPCL, the fluorescence intensity of DPBF caused by SPPCL-*b*-PEO dropped slowly, In other words, the singlet oxygen production ability of SPPCL-PEO can be well controlled by irradiation time, which proves this amphiphilic copolymer SPPCL-PEO could be a promising material in PDT.[29]

In Vitro Drug Release of Doxorubicin-Loaded Nanoparticles

As a most common anticancer drug in clinical application,[30] Doxorubicin (DOX) was loaded in these copolymers by the dialysis method. Our results showed that the DOX-loading capacity of SPPCL-PEO was about 13.8% when a feed ratio of DOX to copolymer of 1 : 5 was employed. To mimic the physiological and endosome/lysosome micro-environments, the *in vitro* release behavior of DOX-loaded micelles in two different buffered solutions (pH 7.4 and pH 5.5) at 37°C was studied (Figure 10). The two-phase-release profile was observed in both buffered solutions. Relatively rapid release in the first phase was observed after10 h, followed by a sustained and slower release

over a prolonged period of time. In comparison with drug release at pH 5.5, the DOX release from the micelle at pH 7.4 was slower, indicating that the DOX-loaded micelles of SPPCL-*b*-PEO are more stable at neutral conditions. This pH-dependent releasing behavior of drugs is of particular interest for tumor-targeted drug delivery using polymeric micelles. In this case, DOX release can be anticipated after micelles are internalized in tumor cells via the endocytosis pathway and entrapped in the acidic endosomal/lysosomal compartments. Consequently, enhanced bioavailability of the DOX is essential for causing cancer cells death.

CONCLUSIONS

In this study, a serial of star-shaped porphyrin-core amphiphilic copolymer SPPCL$_{15}$-*b*-PEO$_{2000}$, SPPCL$_{15}$-*b*-PEO$_{2000}$, SPPCL$_{24}$-*b*-PEO$_{5000}$, and SPPCL15-*b*-PEO$_{5000}$ were well synthesized and thoroughly characterized by NMR, GPC, TEM, and DLS. Fluorescence spectrum shows that the SPPCL-*b*-PEO could produce $^1O_2$, which is very important in PDT. Moreover, the amphiphilic copolymers could self-assemble to form micelles used for photosensitizing agents and further encapsulate hydrophobic drugs such as DOX. In addition, our results proved that the drug release showed pH-induced profile. Therefore, it is a promising technique used the amphiphilic copolymers for delivery system, which could combine photodynamic therapy and chemotherapy for the treatment of cancer.

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REFERENCES

1. Liang, X.; Li, X.; Yue, X.; Dai, Z. *Angew. Chem.* **2011**, *123*, 11826.
2. Hollingsworth, J. V.; Richard, A. J.; Vicente, M. G. a. H.; Russo, P. S. *Biomacromolecules* **2011**, *13*, 60.
3. Zhang, B.; Wang, D.; Li, M.; Li, Y.; Chen, X. *J. Appl. Polym. Sci.* **2012**, *126*, 2067.
4. Lovell, J. F.; Liu, T. W.; Chen, J.; Zheng, G. *Chem. Rev.* **2010**, *110*, 2839.
5. Dosselli, R.; Tampieri, C.; Ruiz-Gonzalez, R. N.; De Munari, S.; Ragàs, X.; Sańchez-Garcĺa, D.; Agut, M.; Nonell, S.; Reddi, E.; Gobbo, M. *J. Med. Chem.* **2013**, *56*, 1052.
6. Lin, S.-T.; Fuchise, K.; Chen, Y.; Sakai, R.; Satoh, T.; Kakuchi, T.; Chen, W.-C. *Soft Matter* **2009**, *5*, 3761.
7. Choi, J.; Ruiz, C. R.; Nesterov, E. E. *Macromolecules* **2010**, *43*, 1964.
8. Takeuchi, Y.; Ichikawa, K.; Yonezawa, S.; Kurohane, K.; Koishi, T.; Nango, M.; Namba, Y.; Oku, N. *J. Control Release* **2004**, *97*, 231.
9. Bechet, D.; Couleaud, P.; Frochot, C.; Viriot, M.-L.; Guillemin, F.; Barberi-Heyob, M. *Trends Biotechnol.* **2008**, *26*, 612.
10. Peng, C.-L.; Shieh, M.-J.; Tsai, M.-H.; Chang, C.-C.; Lai, P.-S. *Biomaterials* **2008**, *29*, 3599.
11. Cai, C.; Wang, L.; Dong, C. M. *J. Polym. Sci. Part A: Polym. Chem.* **2006**, *44*, 2034.
12. Dai, X.-H.; Dong, C.-M.; Yan, D. *J. Phys. Chem. B* **2008**, *112*, 3644.
13. Wang, Y.-C.; Tang, L.-Y.; Sun, T.-M.; Li, C.-H.; Xiong, M.-H.; Wang, J. *Biomacromolecules* **2007**, *9*, 388.
14. Yuan Xiong, X.; Chiu Tam, K.; Huat Gan, L. *J. Appl. Polym. Sci.* **2006**, *100*, 4163.
15. Hecht, S.; Vladimirov, N.; Frechet, J. M. *J. Am. Chem. Soc.* **2001**, *123*, 18.
16. Hsu, C.-Y.; Nieh, M.-P.; Lai, P.-S. *Chem. Commun.* **2012**, *48*, 9343.
17. Dai, X.-H.; Dong, C.-M.; Fa, H.-B.; Yan, D.; Wei, Y. *Biomacromolecules* **2006**, *7*, 3527.
18. Dai, X. H.; Liu, W.; Huang, Y. F.; Dong, C. M. *Adv. Mater. Res.* **2011**, *239*, 1703.
19. Hua, C.; Peng, S.-M.; Dong, C.-M. *Macromolecules* **2008**, *41*, 6686.
20. Quaglia, F.; Ostacolo, L.; Nese, G.; Canciello, M.; De Rosa, G.; Ungaro, F.; Palumbo, R.; La Rotonda, M. I.; Maglio, G. *J. Biomed. Mater. Res. Part A* **2008**, *87*, 563.
21. Ren, T.; Wang, A.; Yuan, W.; Li, L.; Feng, Y. *J. Polym. Sci. Part A Polym. Chem.* **2011**, *49*, 2303.
22. Zhou, W.; Dai, X. H.; Dong, C. M. *Macromol. Biosci.* **2008**, *8*, 268.
23. Wilhelm, M.; Zhao, C. L.; Wang, Y.; Xu, R.; Winnik, M. A.; Mura, J. L.; Riess, G.; Croucher, M. D. *Macromolecules* **1991**, *24*, 1033.
24. Sun, L.; Shen, L. J.; Zhu, M. Q.; Dong, C. M.; Wei, Y. *J. Polym. Sci. Part A: Polym. Chem.* **2010**, *48*, 4583.
25. Ideta, R.; Tasaka, F.; Jang, W.-D.; Nishiyama, N.; Zhang, G.-D.; Harada, A.; Yanagi, Y.; Tamaki, Y.; Aida, T.; Kataoka, K. *Nano Lett.* **2005**, *5*, 2426.
26. de Loos, F.; Reynhout, I. C.; Cornelissen, J. J.; Rowan, A. E.; Nolte, R. *J. Chem. Commun.* **2005**, 60.
27. Discher, D. E.; Ahmed, F. *Annu. Rev. Biomed. Eng.* **2006**, *8*, 323.
28. Haag, R. *Angew. Chem. Int. Ed.* **2004**, *43*, 278.
29. Choi, K.-H.; Wang, K.-K.; Shin, E. P.; Oh, S.-L.; Jung, J.-S.; Kim, H.-K.; Kim, Y.-R. *J. Phys. Chem. C* **2011**, *115*, 3212.
30. Gillies, E. R.; Fréchet, J. M. *Bioconjugate Chem.* **2005**, *16*, 361.