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ARTICLE TYPE

"Breathing" Unimolecular Micelles Based on Novel Star-like Amphiphilic Hybrid Copolymer

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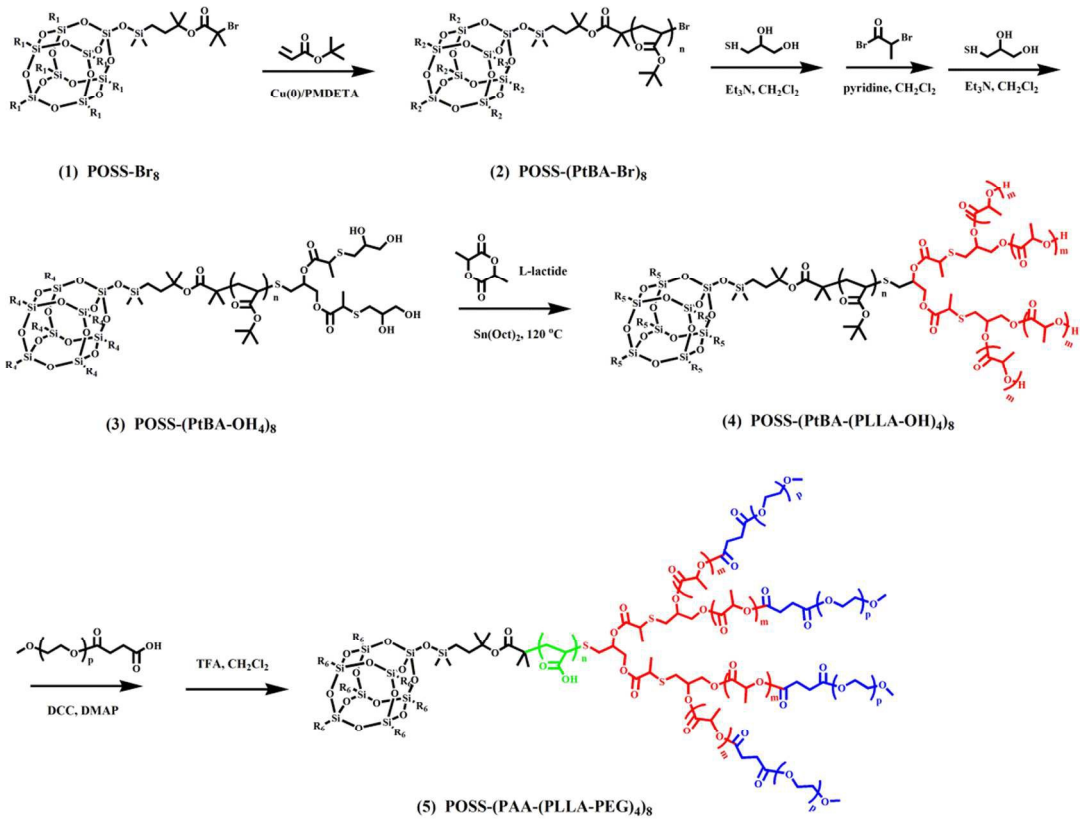
Presented in this paper is a novel unimolecular micell that possesses a pH-induced "breathing" feature. The hybrid copolymer POSS-(PAA-(PLLA-PEG)$_4$)$_8$, in which eight linear-dendritic like arms poly(acrylic acid)-(poly(L-lactide)-poly(ethylene glycol))$_4$ (PAA-(PLLA-PEG)$_4$) are grafted onto an oligomeric silsequioxane core, was synthesized via the combination of SET-LRP, ROP and thio-bromo "click" reaction. ^{1}H NMR confirmed that the formed micelles was composed of a biocompatible PEG outer corona, biodegradable hydrophobic PLA layer in the middle and inner hydrophilic PAA cavities. And, TEM result revealed that the morphology of the self-assembled micelles takes rod-like. Interestingly, the formed micelles can "breathe", that is, size of the micelles changed as pH values varying. Doxorubicin (DOX), an anticancer drug, was encapsulated into the micelles formed by POSS-(PAA- (PLLA-PEG)$_4$)$_8$ to invaculate drug release profile. Due to the unique architecture and properties, this novel amphiphilic hybrid copolymer can be considered a good candidate for controlled drug delivery.

Introduction

Nano-sized polymeric micelles self-assembled from amphiphilic block copolymers have received tremendous attention due to their great promising applications in drug delivery.[1-7] As drug carrier, the inner hydrophobic core can solubilize the hydrophobic drugs, whereas the outer shell protects the loaded drugs from the uptake by the reticuloendothelial system (RES) or premature degradation and prolongs their circulation time in vivo.[8,9] As a result, polymeric micelles can be used as efficient carrier to enhance the therapeutic efficacy and reduce side effects of therapeutic drugs, especially those with poor solubility.[10] However, classical polymeric micelles represent thermodynamic aggregations of amphiphilic macromolecules above their critical micelle concentration (CMC), and they will disassemble into free polymeric chains once the polymer concentration is below CMC. This drawback will heavily hinder their in vivo drug delivery application.[11] In sharp contrast to multimolecular micelles, polymeric micelles from unimolecular amphiphilic copolymers can maintain excellent micellar stability, in which the hydrophilic and hydrophobic segments are covalently linked together. So, well-defined unimolecular amphiphilic block copolymers will be of great interest for the drug delivery applications.

Rapid development of diverse controlled living polymerization techniques[12-16] and emergence of high efficient coupling techniques[17-22] have open pathways to a library of complicated polymers with different compositions and architectures. Among them, star-like (co)polymers is a special class of complex polymers, in which a number of polymer arms are linked covalently to a central core.[23-28] In recent years, multi-arm star-like amphiphilic block copolymers have attracted special interest from researcher due to the fact that they can self-assemble into mono-disperse and stable unimolecular micelles in aqueous solution.[29-36] For example, Song and his colleagues have prepared amphiphilic star-like block copolymer with multi polylactide-b-poly(ethylene glycol) arms using hyperbranched polyester as core.[30]Lin et al reported the synthesis of amphiphilic star-like block copolymer with multi poly(acrylic acid)-b-polystyrene arms based on β-cyclodextrin.[31] However, micelles from amphiphilic multi-arm star-like block copolymers are relatively less studied because of limited model copolymers. Due to their unique architecture and properties, design and synthesis of well-defined amphiphilic multi-arm star-like block copolymers will still be a fascinating subject for polymeric chemists.

Herein, a novel star-like amphiphilic hybrid copolymer has been reported for the first time, in which the arms of linear-dendritic like amphiphilic block copolymers PAA-b-(PLLA-b-PEG)$_4$ are linked to a inorganic polyhedral oligomeric silsequioxane (POSS) core, as shown in Scheme 1. Due to its unique architecture, this copolymer can self-assemble into unimolecular micelles. The biocompatible PEG chain is designed as the hydrophilic outer corona to obtain "stealth micelles". The middle hydrophobic membrane is composed of biodegradable PLA. And the pH-responsive PAA is designed as the inner corona, offering the loading and stabilization of hydrophilic drugs (such as doxorubicin hydrochloride (DOX.HCl)) via electrostatic interaction.[37-40] Like conventional polymeric micelles, this kind of micelles can be explored for controlled release of hydrophilic and hydrophobic drugs. However, it should be noted that the unique micelles based on POSS-(PAA-(PLLA-PEG)$_4$)$_8$ copolymer is more stable in vivo as compared with micelles derived from conventional di- or tri-block copolymers.



Scheme 1. Synthetic route to a novel amphiphilic multi-arm star-like block copolymer POSS-(PAA-(PLLA-b-PEG)$_4$)$_8$.

Experimental Section

Materials

tert-Butyl acrylate (t-BA) (98%, Sigma) was purified by passing through a short column with neutral alumina oxide just before use to remove the inhibitor. Octa(3-hydroxy-3-methylbutyldimethylsiloxy)-POSS was purchased from Hybrid Plastics. L-lactide monomers (99.5%) were purchased from Purac and recrystallized in ethyl acetate before use. Poly(ethylene glycol) methyl ether (M_n=5000) was purchased from Sigma. 2-Bromoisobutyryl bromide (98%, Aldrich), 2-bromopropionyl bromide (97%, Aldrich), N, N, N', N'', N''-pentamethyldiethylenetriamine (PMDETA), copper powder (~625 mesh, Alfa Aesar), thioglycerol (≥ 97%, Aldrich), Tin(II) 2-ethylhexanoate (Sn(Oct)2, 92.5-100%, Aldrich), 4-dimethylamino pyridine (DMAP) (>99%, Aldrich), dicyclohexylcarbodiimide (DCC) (99%, Alfa Aesar) and trifluoroacetic acid (TFA, 99%, Sigma) were used as received. Triethylamine, pyridine and chloroform were dried with calcium hydride and distilled before use. Initiator POSS-Br$_8$ was synthesized according to the literature procedure.[41]

Characterization

^{1}H nuclear magnetic resonance (^{1}H NMR) spectra were recorded on a Bruker Ultrashield 600 MHz/54 mm NMR spectrometer at room temperature using CDCl3 or DMSO-d6 as the solvent. Gel permeation chromatographic (GPC) analysis was performed on a Waters 2690 equipped with an evaporative light scattering detector (Waters 2420) and three phenomenex linear 5 mm styragel columns (500, 104 and 106 Å). DMF was used as eluent at a rate of 1.0 mL min^{-1}. Monodispersed poly(methyl methacrylate) (PMMA) was used as standards. Fourier transform infrared (FT-IR) spectra were recorded on a Perkin Elmer spectrum 2000 spectrometer at a resolution of 1 cm^{-1}. Transmission electron microscopy (TEM) images were obtained on a JEOL JEM 2010F field emission electron microscope operating at an acceleration voltage of 200 kV. The TEM samples were prepared by dropping the polymer solution onto the surface of the carbon-coated 200 mesh copper grid and stained with phosphotungstic acid. The mean size of nanoparticles was determined by dynamic light scattering (DLS) using a Brookhaven BI-9000AT Digital Autocorrelator.

Synthesis of star-like POSS-(PtBA-Br)$_8$ via SET-LRP (2) (Scheme 1) The synthesis process of POSS-(PtBA-Br)$_8$ was conducted under the mild conditions of SET-LRP. Typically, POSS-Br$_8$ (0.656 g, containing Br 1.88 mmol), PMDETA (0.40 mL, 1.88 mmol), tBA (15 mL) and acetone (6 mL) were added to a 50 mL Schlenk flask. The reaction system was degassed by bubbling with nitrogen for 15 min. After Cu(0) powder (0.119g, 1.88 mmol) was introduced into the flask under nitrogen atmosphere, the mixture was purged by nitrogen for another 5 min. Then the flask was immersed into an oil bath maintained at 30 °C. After 1 h, the polymerization was stopped by diluting with THF. The THF solution was passed a short neutral alumina column to remove copper salts. After concentration on rotary evaporator, the residue was precipitated in the mixture of methanol/water (v/v 1:1) two times. The product POSS-(PtBA-Br)$_8$ was dried under vacuum at 40 °C until a constant weight. ($M_{n,NMR}$ = 37,610 g/mol; $M_{n,GPC}$ = 27,150 g/mol, M_w/M_n = 1.46)

Synthesis of POSS-(PtBA-OH$_4$)$_8$ (3) (Scheme 1) Through three steps involving thio-bromo nucleophilic "click" reaction and acylation of hydroxyl groups, POSS-(PtBA-OH$_4$)$_8$ with multi surface hydroxyl groups was obtained from POSS-(PtBA-Br)$_8$. The detail procedure as following: POSS-(PtBA-Br)$_8$ (1.154 g, 32.45 μmol) was dissolved in 5 mL THF, then thioglycerol (0.12 mL, 1.30 mmol) was added. After the solution was cooled to 0 ^{o}C by ice/water bath, Et3N (0.18 mL, 1.30 mmol) was added dropwise into the system via syringe. After 1 h at 0 ^{o}C, the reaction mixture was continuously stirred overnight at room temperature. POSS-(PtBA-2OH)$_8$ was obtained by precipitating the solution in the excessive mixture of methanol/H_2O (v/v 1:1) two times and dried to constant weight under vacuum.

POSS-(PtBA-OH$_2$)$_8$ (0.577 g, 15.84 μmol) was dried by azeotropic distillation with toluene, then 3 mL dry THF and pyridine (0.20 mL, 2.53 mmol) were charged into the flask. After the solution was cooled to 0 oC, 2-bromopropionyl bromide (0.27 mL, 2.53 mmol) was added dropwise via syringe. The reaction lasted for 1 h at 0 ^{o}C and another 24 h at room temperature. The solution was precipitated in the excessive mixture of methanol/H_2O (v/v 1:1) two times to provide the polymer POSS-(PtBA-Br$_2$)$_8$.

POSS-(PtBA-OH$_4$)$_8$ was obtained via once thio-bromo "click" reaction of POSS-(PtBA-Br$_2$)$_8$ with thioglycerol.

Synthesis of POSS-(PtBA-(PLLA-OH)$_4$)$_8$ (4) (Scheme 1) POSS-(PtBA-OH$_4$))$_8$ (0.464 g, 12.09 μmol) was dissolved in 20 mL dry toluene and dried by distilling portion of toluene. Under nitrogen atmosphere, L-lactide (1.038 g, 9.11 mmol) and the 1 mL toluene solution of Sn(Oct$_2$) (0.016 g, 38.70 μmol) were added to the system. The reaction was carried out at 125 ^{o}C for 24 h. POSS-(PtBA-(PLLA-OH)$_4$)$_8$ was obtained by pouring the solution into excessive ethanol and dried under vacuum at 45 ^{o}C. ($M_{n,NMR}$ = 120,750 g/mol; M_{GPC} = 78,560 g/mol, M_w/M_n = 1.21)

Preparation of carboxyl-terminated mPEG (mPEG-COOH) mPEG$_{5k}$-OH (6.420 g, 1.28 mmol), succinic anhydride (0.257 g, 2.56 mmol), DMAP (0.313 g, 2.56 mmol) were dissolved in 200 mL dry chloroform, and the reaction was performed at 80 ^{o}C overnight. The solution was washed with a diluted HCl solution and water successively, then dried over anhydrous $MgSO_4$. The filtrate was concentrated on rotary evaporator and precipitated in diethyl ether two times.

Synthesis of POSS-(PAA-(PLLA-PEG)$_4$)$_8$ (5) (Scheme 1) The target amphiphilic copolymer POSS-(PAA-(PLLA-OH)$_4$)$_8$ was obtained by coupling POSS-(PtBA-(PLLA-OH)$_4$)$_8$ with mPEG-COOH, followed by selective hydrolysis of PtBA blocks using TFA. First, POSS-(PtBA-(PLLA-OH)$_4$)$_8$ (0.412g, 3.56 μmol) and mPEG-COOH (0.925 g, 0.19 mmol) were charged into a flask and dried by azeotropic distillation. After 20 mL dry chloroform was added, the mixture was violently stirred until the solid disappeared completely. Then, DCC (0.074 g, 0.37 mmol) and DMAP (0.045 g, 0.37 mmol) were added into the reaction system and the reaction was allowed to conduct for 24 h under nitrogen atmosphere. The insoluble byproduct dicyclohexylcarbodiurea was removed by filtration. The crude product was purified by slowly adding hexane to the filtered solution until the precipitate appeared and this procedure was repeated two times. The product POSS-(PtBA-(PLLA-OH)$_4$)$_8$ was dried under vacuum at 40 ^{o}C until a constant weight. ($M_{n,NMR}$ = 250,750 g/mol; $M_{n,GPC}$ = 131,670 g/mol, M_w/M_n = 1.29)

The copolymer POSS-(PtBA-4(PLLA-PEG))$_8$ (0.501 g, containing 0.49 mmol tert-butyl ester group) was dissolved in 10 mL CH_2Cl_2, then trifluoroacetic acid (0.94 mL, 12.25 mmol) was added. The reaction mixture was stirred at room temperature for 3 h. After the hydrolysis was completed, the resulting star-like copolymer, POSS-(PAA-(PLLA-PEG)$_4$)$_8$ was purified by precipitating in excessive hexane two times.

Preparation of DOX- loaded micelles from POSS-(PAA-(PLLA-PEG)$_4$)$_8$ star-shaped micelles The blank and DOX-loaded POSS-(PAA-(PLLA-PEG)$_4$)$_8$ micelles were prepared according to the emulsion method. In a typical experiment, POSS-(PAA- (PLLA-PEG)$_4$)$_8$ (40 mg) were dissolved in 40 mL of deionized water, while 8 mg DOX were dissolved in chloroform/TEA mixture and stirred for 4 h at room temperature until fully dissolved. Then the DOX/ chloroform solution was added dropwise into the polymer solution and subsequently stirred at room temperature for 48 hours to vaporize chloroform fully. The remaining DOX residuals, together with blank and DOX-loaded micelles were centrifuged to remove excess DOX residuals, filtered using a membrane filter (0.80 μm pore) to remove aggregated particles and subsequently freeze-dried to obtain the final product which was stored at −20 °C until further experiments.

The DOX loading content (LC) and entrapment efficiency (EE) were determined by fluorescence. One milligram of the DOX-loaded micelle powder was dissolved in 10 mL of DMSO. The concentration of DOX at 590 nm was recorded with reference to a calibration curve of pure DOX/DMSO solution. The LC and EE of DOX were calculated using the following formulas, respectively.

$$\mathrm{LC}(\%) = \frac{mass\ of\ drug\ encapsulated\ in\ micelles}{mass\ of\ drug-loaded\ micelles} \times 100\%$$

$$\mathrm{EE}(\%) = \frac{mass\ of\ drug\ loaded\ in\ micelles}{mass\ of\ drug\ initially\ added} \times 100\%$$

***In Vitro* Release of DOX from Micelles**

The *in vitro* DOX release properties from the POSS-(PAA-(PLLA-PEG)$_4$)$_8$ self-assembled micelles were determined as follows: 2 mg of DOX-entrapped micelle was suspended in 1 mL of PBS solvent and then placed in a pre-swollen cellulose dialysis membrane tube (MWCO 5000). The dialysis tube was then immersed into 40 mL of PBS buffer at pH7, and kept in a 37 °C water bath. At specific time intervals, a 2 mL (V_e) sample was taken out and replaced by 2 mL fresh buffer(with different pH) to maintain the total volume. The concentrations of DOX in different samples were determined using fluorescence. The cumulative drug release percent (E_r) was calculated based on the equation

$$E_r(\%) = \frac{V_e \sum_1^{n-1} C_1 + V_0 C_n}{m_{DOX}} \times 100$$

where m_{DOX} represents the amount of DOX in the micelle (mg), V_0 is the whole volume of the release media (V_0 = 40 mL), and C_n represents the concentration of DOX in the *n*th sample (mg). The *in vitro* experiments were repeated three times, and all samples were analyzed in triplicate to get the final release curves.

Result and discussion

Synthesis and Characterization of star-like amphiphilic copolymer POSS-(PAA-4(PLLA-PEG))$_8$ The star-like amphiphilic copolymer with POSS core and linear-dendritic like

PAA- $(PLLA\text{-}PEG)_4$ arms was successfully synthesized according to the steps shown in Scheme 1. The star-like polymer POSS-$(PtBA\text{-}Br)_8$ was first synthesized by SET-LRP of t-BA monomers using POSS-Br_8 as initiator and Cu(0)/PMDETA as catalyst. Figure 2A shows the GPC trace of POSS-$(PtBA\text{-}Br)_8$, which is a monomodal peak, indicating that POSS-Br_8 successfully initiated polymerization of tBA monomer. ^{1}H NMR spectrum of the obtained POSS-$(PtBA\text{-}Br)_8$ is shown in Fig 1A. The peaks at about 0.10 (a) and 2.23 (c) ppm are attributed to methyl protons of the initiator adjacent to the core and methine protons in PtBA arms, respectively. The molecular weight of POSS-$(PtBA\text{-}Br)_8$ is derived from the ^{1}H NMR spectrum following the formula (1):

$$M_{n,NMR} = \frac{6A_c}{A_a} \times 128 \times 8 + 2795 \quad (1)$$

where A_a and A_c stand for the integral areas of the peaks (a) and (c). The values of 128 and 2795 stand for the molecular weight of tBA repeating units and the initiator, respectively. It should be noted that the molecular weight of POSS-$(PtBA\text{-}Br)_8$ based on GPC is different from that of ^{1}H NMR (Table 1). This is because that the structure of POSS-$(PtBA\text{-}Br)_8$ is star-like, however, the standard of GPC is the linear PMMA.

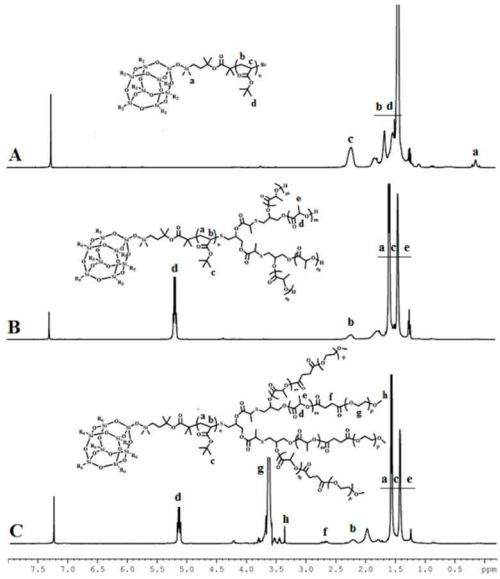


Fig 1. ^{1}H NMR spectra of POSS-$(PtBA\text{-}Br)_8$ (A), POSS-$(PtBA\text{-}(PLLA\text{-}OH)_4)_8$ (B) and POSS-$(PtBA\text{-}(PLLA\text{-}PEG)_4)_8$ (C) in $CDCl_3$.

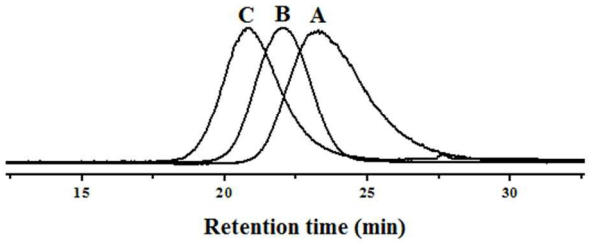


Figure 2. GPC traces of POSS-$(PtBA\text{-}Br)_8$ (A), POSS-$(PtBA\text{-}(PLLA\text{-}OH)_4)_8$ (B) and POSS-$(PtBA\text{-}(PLLA\text{-}PEG)_4)_8$ (C) with DMF as eluent.

Thio-bromo nucleophilic substitution reaction reported by Percec was rapid and proceeded with near-quantitative conversion, possessing characteristics of "click" reactions.[42-45] Meanwhile, this reaction was shown to be compatible with a range of functional thiols. Through iterative three steps involving thio-bromo "click" reaction of thioglycerol with α-bromoester and acylation of hydroxyl groups with 2-bromopropionyl bromide, POSS-$(PtBA\text{-}OH_4)_8$ with multi surface hydroxyl groups was obtained from POSS-$(PtBA\text{-}Br)_8$. ^{1}H NMR technique was used to analyze the end group transformation processes. As compared to that of POSS-$(PtBA\text{-}Br)_8$ (Figure 3A), the signal at 4.15 ppm assigned to methine protons neighboring to the terminal bromide groups disappeared and the characteristic signals of thioglycerol at 3.56~3.73 ppm could be observed in the spectrum of POSS-$(PtBA\text{-}OH_2)_8$ (Figure 3B), conforming that the thio-bromo reaction was performed completely. After acylation of –OH on POSS-$(PtBA\text{-}OH_2)_8$, the signals at 3.56~3.73 ppm belonging to thioglycerol shifted entirely to lower resonance field (4.40 ppm (g) and 5.03 ppm (f)), indicating that this reaction was complete (Figure 3C). From Figure 3D, it can be seen that the signal ascribed to methine proton of 2-bromo-propanoyl groups disappeared completely and the characteristic signals of thioglycerol at 3.56~3.73 ppm could be observed again after another thio-bromo "click" reaction of POSS-$(PtBA\text{-}Br_2)_8$ with thioglycerol. Based on the results above, it can be concluded that POSS-$(PtBA\text{-}OH_4)_8$ was successfully obtained.

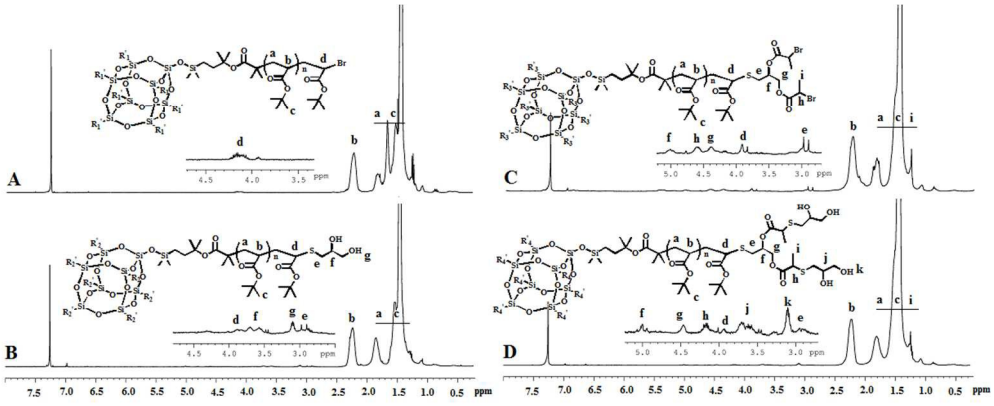


Figure 3. ^{1}H NMR spectra of POSS-$(PtBA\text{-}Br)_8$ (A), POSS-$(PtBA\text{-}OH_2)_8$ (B), POSS-$(PtBA\text{-}Br_2)_8$ (C) and POSS-$(PtBA\text{-}OH_4)_8$ (D) in $CDCl_3$.

Table 1. Characterization of the star-like amphiphilic copolymer POSS-(PAA- (PLLA-PEG)$_4$)$_8$.

Sample	Length of Arms			Coupling Efficiency (%) [b]	$M_{n,NMR}$ [c]	$M_{n,GPC}$ [d]	M_w/M_n [d]
	PtBA[a]	PLLA[a]	PEG				
POSS-(PtBA-Br)$_8$	4,350				37,610	27,150	1.46
POSS-(PtBA-(PLLA-OH)$_4$)$_8$	4,350	2,510			120,750,	78,560	1.21
POSS-(PtBA-(PLLA-PEG)$_4$)$_8$	4,350	2,510	5,000	81.2	250,750	131,670	1.29

[a] Determined by ^{1}H NMR spectroscopy.
[b] Coupling efficiency between POSS-(PtBA-(PLLA-OH)$_4$)$_8$ and mPEG-COOH, determined by ^{1}H NMR spectroscopy.
[c] Calculated according to the formulas (1), (2) and (3), respectively.
[d] Determined by GPC analysis with PMMA as standard.

Using POSS-(PtBA-OH$_4$)$_8$ as macroinitiator initiated ring-opening polymerization (ROP) of L-lactide to provide the copolymer POSS-(PtBA-(PLLA-OH)$_4$)$_8$. The GPC trace of POSS-(PtBA-(PLLA-OH)$_4$)$_8$ is shown in Figure 2B, which was monomodal and clearly shifted toward the higher molecular region as comparing with the precursor POSS-(PtBA-Br)$_8$. The FT-IR spectrum of POSS-(PtBA-(PLLA-OH)$_4$)$_8$ is given in Figure 4B, the characteristic absorption at 1756 cm^{-1} corresponds to the ester carbonyl of PLLA chains. Meanwhile, the peaks (d) at 5.14 and (e) at 1.56 ppm assigned to the methine and methyl protons of the PLA block could be observed clearly in the ^{1}H NMR spectrum of POSS-(PtBA-b-(PLLA-OH)$_4$)$_8$ (Figure 1B). These results conformed the successful synthesis of PtBA-b-(PLLA-OH)$_4$. The repeat units of the PLLA chains were derived from the intensity ratio of the peaks (b) at 2.23 to (d) at 5.14 ppm. And, the molecular weight of POSS-(PtBA-b-(PLLA-OH)$_4$)$_8$ was calculated according to the following formula (2):

$$M'_{n,NMR} = M_{n,NMR} + \frac{34A_d}{4A_b} \times 32 \times 72 \quad (2)$$

where $M'_{n,NMR}$ and $M_{n,NMR}$ stand for the molecular weight of POSS-(PtBA-b-(PLLA-OH)$_4$)$_8$ and POSS-(PtBA-OH$_4$)$_8$, respectively; A_d and A_b stand for the integral area of the peaks (d) and (b), respectively; The value 32 stands for the number of PLLA chains and 72 stands for the molecular weight of the repeat unit of PLLA. The result is listed in Table 1.

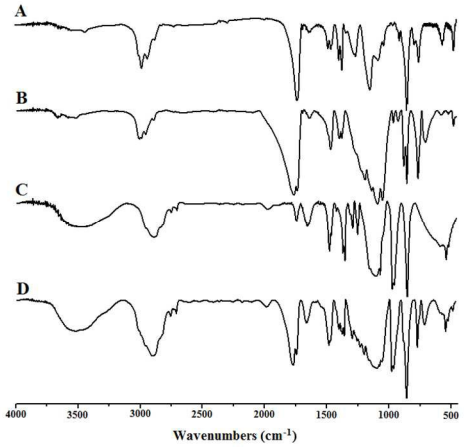


Figure 4. FT-IR spectra of POSS-(PtBA-Br)$_8$ (A), POSS-(PtBA-(PLLA-OH)$_4$)$_8$ (B), mPEG-COOH (C) and POSS-(PtBA-(PLLA-PEG)$_4$)$_8$ (D).

Star-like amphiphilic copolymer was obtained via the coupling reaction of POSS-(PtBA-b-(PLLA-OH)$_4$)$_8$ with mPEG-COOH in the presence of DCC and DMAP. POSS-(PtBA-b-(PLLA-OH)$_4$)$_8$ coupling successfully with mPEG-COOH was proved by GPC, FT-IR and ^{1}H NMR. The GPC trace of POSS-(PtBA-b-(PLLA-PEG)$_4$)$_8$ (Figure 2C) moves toward the higher molecular region as compared with the precursor. From FT-IR spectrum POSS-(PtBA-b-(PLLA-PEG)$_4$)$_8$ (Figure 4D), it can be seen that the intensity of the peak at 2890 cm^{-1} increased greatly after coupling reaction, which is due to the C-H stretching band of PEG block. In the ^{1}H NMR spectrum of POSS-(PtBA-(PLLA-PEG)$_4$)$_8$, the new signals (g) at 3.65 ppm and (h) at 3.37 ppm attributed to methylene and methyl protons of PEG block could be observed clearly (Figure 1C). According to the relative integral values of the methylene protons (g) of PEG and methine protons (d) of PLA blocks, the coupling efficiency can be easily measured and the result is about 81.2%. The molecular weight of POSS-(PtBA-b-(PLLA-PEG)$_4$)$_8$ is determined based on the following formula (3):

$$M''_{n,NMR} = M'_{n,NMR} + 5000 \times 32 \times E \quad (3)$$

where $M''_{n,NMR}$ and $M'_{n,NMR}$ stand for the molecular weight of POSS-(PtBA-b-(PLLA-PEG)$_4$)$_8$ and POSS-(PtBA-(PLLA-OH)$_4$)$_8$, respectively; The value 5000 stands for the molecular weight of PEG; E stands for the efficiency of coupling. The result is listed in Table 1.

Under the presence of TFA, selective hydrolysis of PtBA block provided the targeted star-like amphiphilic copolymer POSS-(PAA-b-(PLLA-PEG)$_4$)$_8$. Figure 5 A shows the ^{1}H NMR spectrum of POSS-(PAA-b-(PLLA-PEG)$_4$)$_8$, in which intensity of the resonance signal at 1.20 ppm decreased significantly as compared with that of the precursor POSS-(PtBA-b-(PLLA-PEG)$_4$)$_8$, indicating the removal of terbutyl groups on the PtBA block.

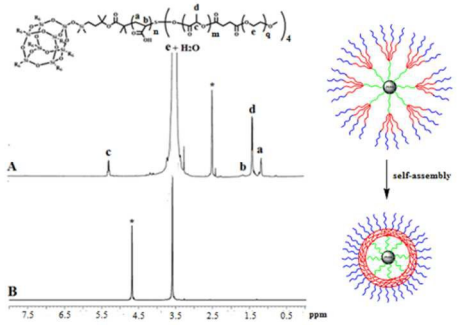


Figure 5. ^{1}H NMR spectrum of POSS-(PAA-(PLLA-PEG)$_4$)$_8$ in DMSO-d$_6$ (A) and D$_2$O (B).

Properties of unimolecular micelles formed by star-like amphiphilic copolymer POSS-(PAA-b-(PLLA-PEG)$_4$)$_8$

The morphology and size of the formed micelles were analyzed using TEM and DLS. The TEM image (Figure 6A) shows that this unimolecular amphiphilic copolymer self-assembled into well-dispersed rod-like micelles with a size about 100 nm in 1.0 mg mL^{-1} aqueous solution and this value is basically consistent

with the theoretical size (110 nm). The average size of the micelles determined by DLS was about 130 nm. It should be noted that the size measured by DLS was bigger than that measured by TEM because DLS measures the hydrodynamic size of the nanoparticles, while TEM measures the size of the dried nanoparticles. At the same time, we investigated the properties of the micelles as the concentration of the solution decreased from 1.0 mg mL^{-1} to 0.05 mg mL^{-1}. From Figure 6B, it can be seen that the morphology and size of the micelles didn't change as the solution was diluted 20 times. Meanwhile, the size of the copolymer POSS-(PAA-(PLLA-PEG)$_4$)$_8$ was also characterized in THF. Due to the fact that THF is a good solvent for POSS-(PAA-(PLLA-PEG)$_4$)$_8$, it should exist as separate unimolecules. The obtained average size is about 154 nm, which is bigger than that (~ 130 nm) determined in aqueous solution. The reason is that the middle PLLA chains are stretching in THF good solvent, however, they aggregate in aqueous solution. So, it can be basically concluded that the formed micelles were unimolecular.

According to the unique structure, the amphiphilic copolymer POSS-(PAA-(PLLA-PEG)$_4$)$_8$ could self-assemble into the micelles having a biodegradable PLA membrane with the biocompatible PEG outer corona and PAA hydrophilic cavity. Its structure was confirmed by the ^{1}H NMR technique. From Figure 5, it can be seen that the characteristic signals belonging to PLA and PAA segments can be observed clearly in DMSO-d_6, however, they disappeared completely in D_2O. This phenomenon demonstrated the formation of the micelles with the PEG chains stretching on the exterior of the PLA membrane and PAA chains on its interior. Otherwise, the signal of PAA should be also observed in D_2O media.

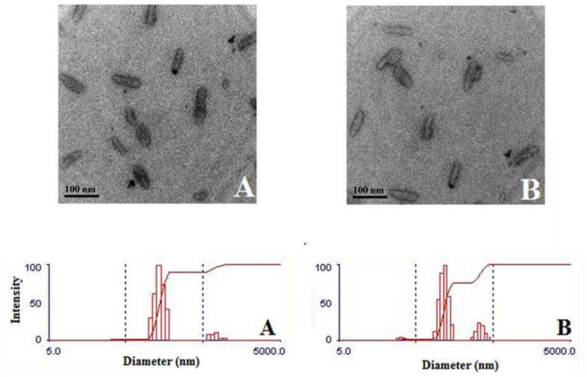


Figure 6. TEM images of the micelles formed from star-like amphiphilic copolymer POSS-(PAA-(PLLA-PEG)$_4$)$_8$ with different concentrations (A) 1 mg/mL and (B) 0.05 mg/mL.

It is well known that PAA is one of the most studied pH-responsive polymers, and micelles with hydrophilic PAA outer corona displayed size-change behaviors in the presence of pH change. So, it can be hypothesized that micelles with inner hydrophilic PAA cavity should display swelling-deswelling behavior with varying of the system pH. Swelling the formed micelles as a function of pH is shown in Figure 7. It is apparent from the graph that the micelles were minimum at pH about 4 when the PAA was not charged. As pH increased, the micelles progressively swelled due to the increasing amount of ionized carboxylic groups. At pH about 7 the swelling reached a maximum since the carboxylic groups were completely deprotonated. However, the micelless began to deswell at pH above 7, which might be contributed to the electrostatic attraction exerted from the charged carbox-ylate and counter ions. The fact indicated that the micelless can "breathe" with variation of the pH.

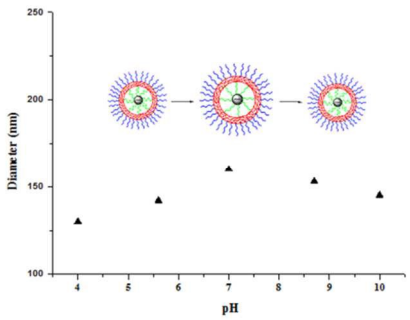


Figure 7. pH-dependent size of the micelles formed by the hybrid copolymer POSS-(PAA-(PLLA-OH)$_4$)$_8$.

***In- vitro* release of DOX from POSS-(PAA- (PLLA-PEG)$_4$)$_8$ star-shaped micelles** The unique micelles from POSS-(PAA-(PLLA-PEG)$_4$)$_8$ hold great promising applications in drug delivery area. This is because that the PLLA membrane can load hydrophobic drugs like the traditional core-shell structural micelles. Most importantly, the PAA aqueous core located on the inside of the micelless can be used to efficiently encapsulate some hydrophilic drugs (such as DOX.HCl) via the electrostatic interactions. Hydrophobic DOX was first used to test the in vitro drug encapsulation and release of the formed micelles. The actual DLC and DLE of the POSS-(PAA-(PLLA-PEG)$_4$)$_8$ micelless were 8.37% and 50.22%, respectively, as the theoretical DLC was set to 20%. As shown in Figure 8, approximately 68.5% DOX was released in a controlled manner from the micelless in first 24 hours, which makes this hybrid copolymer particularly appealing for hydrophobic drugs release. The detail studies of hydrophilic DOX.HCl release from the POSS-(PAA-(PLLA-PEG)4)8 micelles under are on-going. The micelles of POSS-(PAA-(PLLA-PEG)4)8 showed apparent loading efficiency toward to DOX.HCl due to the electrostatic interactions between the inner PAA chains and DOX.HCl (see Figure S1 and S2). Meanwhile, the DOX.HCl release might show pH-responsive feature, which relies on the protonation effect of PAA chains at lower pH.

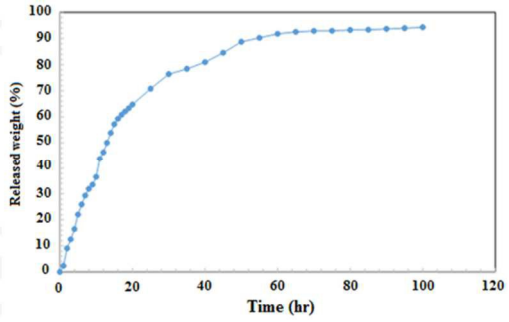


Figure 8. Release Curve of DOX from POSS-(PAA-(PLLA-PEG)$_4$)$_8$ Star-Shaped Micelles.

Conclusion

In summary, a novel unimolecular star-like amphiphilic copolymer POSS-(PAA-(PLLA-PEG)$_4$)$_8$ has been developed for the first time via the combination of SET-LRP, ROP and thio-bromo "click" reaction. Due to its unique architecture, this copolymer self-assembled into special nano-sized unimolecular micelles, which possesses a PLA hydrophobic membrane with the PEG corona and interior PAA hydrophilic cavities. This kind of micelles based on POSS-(PAA-(PLLA-PEG)$_4$)$_8$ copolymer

holds great promising applications in controlled drug delivery due to the following advantages: 1) They possess better stability in vivo than the conventional multimolecular micelles. 2) Their PLA membrane of the micelles can encapsulate some hydrophobic drugs. 3) Their inner PAA hydrophilic cavities can load some hydrophilic drugs via electrostatic interactions, such as DOX·HCl. Moreover, this micelle can be further explored to prepare a range of biomedical materials. For example, it can be used for cancer theranostics as superparamagnetic iron oxide nanoparticles are precipitated in the inner PAA cavities. In one word, the unique architecture and features make this novel amphiphilic copolymer promising materials in the biomedical area.

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