

RSC Advances



This is an *Accepted Manuscript*, which has been through the Royal Society of Chemistry peer review process and has been accepted for publication.

Accepted Manuscripts are published online shortly after acceptance, before technical editing, formatting and proof reading. Using this free service, authors can make their results available to the community, in citable form, before we publish the edited article. This *Accepted Manuscript* will be replaced by the edited, formatted and paginated article as soon as this is available.

You can find more information about *Accepted Manuscripts* in the [Information for Authors](#).

Please note that technical editing may introduce minor changes to the text and/or graphics, which may alter content. The journal's standard [Terms & Conditions](#) and the [Ethical guidelines](#) still apply. In no event shall the Royal Society of Chemistry be held responsible for any errors or omissions in this *Accepted Manuscript* or any consequences arising from the use of any information it contains.



Journal Name

PAPER

Preparation of polymersomes in pure water for facile antibacterial applications

Tao Wang,^a Jinhui Jiang,^a Yufen Xiao,^a Yijie Zou,^a Jingyi Gao,^a and Jianzhong Du^{a,b,*}Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/

We report a facile strategy for preparing persistent and effective antibacterial polymersomes (polymer vesicles) based on triblock copolymers synthesized by sequential copolymerization of 2-diethylaminoethyl methacrylate (DEA) and 2-(*tert*-butylamino)ethyl methacrylate (TA) via atom transfer radical polymerization (ATRP). The poly(ethylene oxide)-*block*-poly(2-diethylaminoethyl methacrylate)-*block*-poly[(2-*tert*-butylamino)ethyl methacrylate] (PEO-*b*-PDEA-*b*-PTA) triblock copolymers can self-assemble into polymersomes in aqueous solution when directly dissolved in pure water without the aid of organic solvents. ¹H NMR and gel permeation chromatography (GPC) studies confirmed the well-defined copolymers. Transmission electron microscopy (TEM) and dynamic light scattering (DLS) studies proved the formation of polymersomes. Antibacterial tests showed good antibacterial activities of polymersomes against both Gram-positive and Gram-negative bacteria. Moreover, those polymersomes may be facilely sprayed in hospitals which are susceptible to bacterial attack for long-term effective antibacterial applications.

Introduction

Amphiphilic block copolymers can self-assemble into a range of nanostructures such as spherical micelles,^{1,2} cylinders and cocoons,³ vesicles⁴⁻⁶ and more complex structures.^{7,8} Generally, solvent-switch method is used for self-assembly,⁹ with which the copolymer is dissolved in a water-miscible organic solvent and water is then added gradually to the solution to induce the formation of nanostructures. However, an organic solvent often needs to be removed by dialysis, which is time consuming and not environmentally benign. Therefore, organic-solvent-free methods such as film rehydration, bulk rehydration, and directly dissolving copolymers in pure water to form nanostructures attracted much attention.¹⁰⁻¹² For some pH- or thermo-responsive block copolymers, the self-assembly process can be achieved through the dissolution of copolymer in pure water followed by the simple adjustment of pH^{13,14} or temperature.¹⁵⁻¹⁷

Recently, more and more attention has been paid to antimicrobial compounds for their broad applications in the field of bio-medicine, food packaging, and sterilization of hygienic areas.¹⁸⁻²⁰ Normally, there are two categories of antimicrobial materials according to their biocidal mechanism: release-killing agents and contact-killing agents.²¹ The release-killing agents can release a low molecular weight biocide.

Typical examples are chlorine-releasing *N*-halamines,²² derivatives of isothazalone,²³ and composites of silver.²⁴ Although these materials boast superior antibacterial capabilities, they have some shortages such as time limitation of antibacterial activities, environmental problems, and the formation of bacterial resistance. In contrast, the contact-killing biocides show antimicrobial activity *via* direct contact with bacteria. Polymers with substituted quaternary ammonium compounds,²⁵ phosphonium salts,^{26,27} and rhodanine derivatives⁸ belong to this category. These polycationic substances have advantages of long-term durability, reduced bacterial resistance, and environmentally friendly performance.

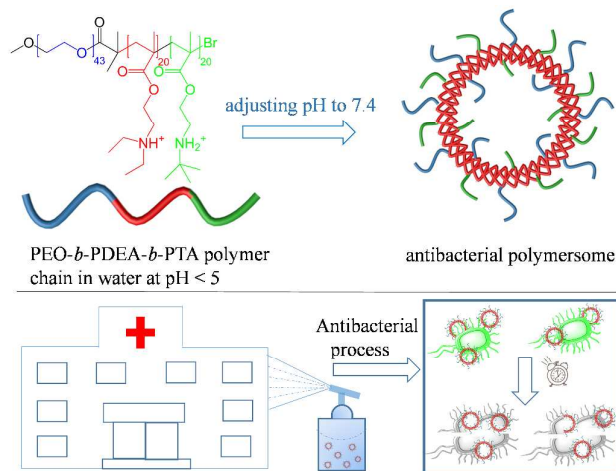
Poly[2-(*tert*-butylamino)ethyl methacrylate] (PTA) is one of these polycationic substances that exhibits a strong antibacterial property and low toxicity to human cells. PTA can penetrate the cell membrane/wall of bacteria for its partially hydrophilic and partially hydrophobic property in neutral water.^{23,28} What's more, the positively charged *tert*-butylamino groups of PTA in neutral water is relatively high,²⁹ which can replace the divalent cations of the outer membrane thus leading to disintegration of the cell membrane and final death of the bacteria.³⁰ Our group recently focused on the preparation of water-dispersible polymersomes that exhibit excellent antibacterial properties.^{31,32} For example, PEO-*b*-PCL-*b*-PTA triblock copolymer micelles have been demonstrated with good antimicrobial efficacy against Gram-negative and Gram-positive bacteria.³³ The micellar nanostructure was confirmed to have better antibacterial efficacy than individual polymer chains.

Herein, we report the formation of water dispersible and antibacterial polymersomes from another kind of triblock

^a Key Laboratory of Advanced Civil Engineering Materials of Ministry of Education, School of Materials Science and Engineering, Tongji University, 4800 Caoan Road, Shanghai 201804, China.

^b Shanghai 10th People's Hospital, Tongji University School of Medicine, 301 Middle Yanchang Road, Shanghai 200072, China.
Email: jzdu@tongji.edu.cn; Fax: +86-21-6958 4723; Tel: +86-21-6958 0239

copolymer, PEO-*b*-PDEA-*b*-PTA. The polymersomes can kill both Gram-negative bacteria such as *Escherichia coli* (*E. coli*) and Gram-positive bacteria such as *Staphylococcus aureus* (*S. aureus*) without quaternary ammonium moieties or loading external antibiotics or nano-silvers (Scheme 1). Since the pH-responsive PDEA (whose pK_a is 7.4) block will become hydrophobic in neutral or alkaline water,⁵ the triblock copolymers can self-assemble into polymersomes in water simply by adjusting the pH from acidic state to neutral state. The long-time stable polymersome solution can potentially function as an antibacterial agent when sprayed in the hospital or similar places which are susceptible to bacterial attack.



Scheme 1. Illustration of PEO-*b*-PDEA-*b*-PTA polymersomes with potential antibacterial applications by spraying on surfaces. The polymersomes consist of PEO and PTA coronas (blue and green) and PDEA membranes (red). These polymersomes can be used as a long-term antibacterial material by spraying on surfaces that vulnerable to bacterial attack.

Experimental Section

Materials

Poly(ethylene oxide) monomethyl ether (PEO) was purchased from Sigma-Aldrich. 2-Diethylaminoethyl methacrylate (DEA) was purchased from Aladdin Industrial Corporation. The DEA monomer was passed through an alumina B column to remove the inhibitor before use. Dibromobutyl bromine was purchased from Aladdin Industrial Corporation. Bipyridine (bpy), 1, 1, 4, 7, 10, 10-hexamethyltriethylenetetramine (HMTETA) and 2-(*tert*-butylamino)ethyl methacrylate (TA) were purchased from J&K Scientific Ltd and dried over CaH_2 overnight, then distilled under reduced pressure before use. $CDCl_3$ was purchased from J&K Scientific Ltd. $Cu(I)Br$, *n*-hexane, methanol, chloroform, acetone, dichloromethane, tetrahydrofuran (THF), and other solvents were purchased from Aladdin Industrial Corporation. $Cu(I)Br$ was stirred in glacial acetic acid for 5 h, then washed with acetone six times and stored with argon protection.

Gram-negative bacterium *E. coli* (ATCC35218) and Gram-positive bacterium *S. aureus* (ATCC29213) were purchased from

Nanjing Bianzhen Biological Technology Co., Ltd. LB Agar and LB broth were purchased from Aladdin.

Synthesis of PEO-Br macroinitiator. The PEO-Br macroinitiator was synthesized according to our previously reported procedures.²⁴ The 1H NMR spectrum is shown in Fig. S1 in ESI†.

Synthesis of PEO-*b*-PDEA-Br macroinitiator. PEO-*b*-PDEA-Br block copolymer was synthesized using a typical ATRP protocol. A flask with a rubber septum and a magnetic stirrer bar was charged with PEO-*b*-PDEA-Br macroinitiator (1.500 g, 0.7407 mmol), bpy (0.2335 μ L, 1.481 mmol), DEA (2.882 g, 15.56 mmol) and anisole (3.50 mL). Then the mixture was deoxygenated by flushing argon for 30 min, followed by the addition of $CuBr$ (0.1079 g, 0.7407 mmol). The mixture was reacted at 60 $^{\circ}C$ under the protection of argon. The reaction was quenched after 16 h by cooling the mixture to room temperature and exposing it to air. The mixture was diluted with THF and the catalyst was removed through a silica gel column. Then the polymer solution was concentrated by the rotary evaporator, and dialyzed against DI water to remove excess monomer and solvent. The final copolymer was obtained by freeze-drying. The 1H NMR spectrum is shown in Fig. S2 in ESI†.

Synthesis of PEO-*b*-PDEA-*b*-PTA triblock copolymer. PEO-*b*-PDEA-*b*-PTA was synthesized by ATRP according to a similar protocol to PEO-*b*-PDEA-Br. First, PEO-*b*-PDEA-Br macroinitiator (1.000 g, 0.1745 mmol), TA (1.018 g, 0.1745 mmol), and HMTETA (75.6 μ L, 0.1745 mmol) were added to a 25 mL of flask with a rubber septum and a magnetic stirrer bar. Then the mixture was deoxygenated by flushing argon for 30 min, followed by the addition of $CuBr$ (0.0327 g, 0.1745 mmol). The mixture was reacted at 70 $^{\circ}C$ with vigorous stirring under the protection of argon. The reaction was polymerized for 24 h and terminated by cooling the solution to room temperature and exposing it to air. The polymer solution was diluted with THF and passed through a silica gel column for the removal of catalyst. Then the mixture was dialyzed against DI water for 72 h and freeze-dried to give a purified copolymer. The 1H NMR spectrum is shown in Fig. 2.

Preparation of PEO-*b*-PDEA-*b*-PTA polymersome. The PEO-*b*-PDEA-*b*-PTA polymersome was prepared according to the following protocol: 15 mg of PEO-*b*-PDEA-*b*-PTA triblock copolymer was first dissolved in 3 mL of DI water (pH 2). Then NaOH solution (pH 11) was added dropwise under vigorous stirring until the pH of the mixture reached 7.4. The PEO-*b*-PDEA-*b*-PTA polymersome solution was characterized by DLS and TEM to determine the hydrodynamic diameter (D_h) and morphology.

Antibacterial test. Two methods were used to test the antibacterial activity of the PEO-*b*-PDEA-*b*-PTA polymersome. Two kinds of bacteria were used, Gram-negative bacterium *E. coli* (ATCC35218) and Gram-positive bacterium *S. aureus* (ATCC29213).

First, the LB broth culture solution (100 μ L) was placed into each well of cells, the polymersome solution (*ca.* 10.0 mg/mL, 100 μ L) was then added to the first cell. The mixture was then diluted to different concentrations (2-fold dilution). The bacterial solution (100 μ L) was then added into each of them. The MICs (Minimal Inhibitory Concentration) were recorded by measuring the optical density absorbance at 600 nm of UV light on a UV-vis spectrophotometer (UV-759S, Q/YXL270, Shanghai Precision & Scientific Instrument Co., Ltd). Broth containing cells alone was used as control and the tests were repeated at least three times.

Alternatively, 10 mL of various polymersome solutions with concentrations of 1.200, 0.600, 0.300, 0.150, 0.075 and 0.038 mM prepared by the serial dilution of the 1.200 mM polymersome solution and culture solution were added into conical flasks, using only culture solution without polymersome as control. Then the *E. coli* microorganism solution (10 μ L) which had an optical density reading of 0.8 at 600 nm wavelength was added. Then 100 μ L of each polymersome and *E. coli* solution was evenly spread on the surfaces of LB agar of the plate. The plate was incubated for 1–2 days at 37 $^{\circ}$ C and the colony forming units were counted. Each concentration was incubated on three plates. The same procedure was repeated when using *S. aureus* as the bacterium.

Characterization

GPC. The molecular weight and polydispersity of PEO-*b*-PDEA-Br and PEO-*b*-PDEA-*b*-PTA was evaluated using a DMF GPC conducted by a Waters Breeze 1525 GPC analysis system with two PL mix-D columns with HPLC grade DMF as the eluent at a flow rate of 1.0 mL/min at 30 $^{\circ}$ C. The polymer was dissolved in DMF and filtered prior to analysis.

$^1\text{H NMR}$. Proton nuclear magnetic resonance ($^1\text{H NMR}$) spectra were recorded using a Bruker AV 400 MHz spectrometer at room temperature in CDCl_3 .

DLS. The D_h and polydispersity of polymersomes in aqueous solution were determined by the dynamic light scattering (DLS). The D_h of polymersomes was characterized by ZETASIZER Nano series instrument (Malvern Instruments ZS 90) at a fixed scattering angle of 90 $^{\circ}$. Data processing was carried out using cumulant analysis of the experimental correlation function and the D_h was calculated from the computed diffusion coefficients using the Stokes–Einstein equation. Each reported measurement was conducted for three runs.

TEM. TEM images were taken with a JEOL JEM-2100F instrument at 200 kV equipped with a Gatan 894 Ultrascan 1 k CCD camera. The polymersome solution was diluted to 400 $\mu\text{g/mL}$ and 10.0 μL of sample was dropped onto the carbon-coated copper grid and evaporated at ambient temperature. The sample was stained by 1.0 wt% phosphotungstic acid aqueous solution.

Results and discussion

Synthesis of PEO-*b*-PDEA-*b*-PTA triblock copolymer.

The PEO-*b*-PDEA-*b*-PTA triblock copolymer was synthesized according to the following steps, as shown in Fig. 1. First, PEO-Br macro-initiator was prepared by esterification of PEO-OH by adding 2-bromoisobutyryl bromide. $^1\text{H NMR}$ spectrum of PEO-Br indicated that the esterification efficiency is over 95%, as shown in Fig. S1 in the ESI † . The PEO-*b*-PDEA-Br diblock copolymer was synthesized through a typical ATRP process, using PEO-Br as the macro-initiator, then serving as the ATRP initiator for the synthesis of the target PEO-*b*-PDEA-*b*-PTA triblock copolymer. The $^1\text{H NMR}$ spectrum of PEO $_{43}$ -*b*-PDEA $_{20}$ -*b*-PTA $_{20}$ was shown in Fig. 2. The degrees of polymerization of DEA and TA were 20 and 20, which were calculated by comparing peak b with peak g or peak n. GPC analysis in Fig. 3 indicated similar results and further gave out the molecular weight and molecular weight distribution of the macro-initiator, PEO $_{43}$ -*b*-PDEA $_{20}$ -Br ($M_w = 3300$, $M_w/M_n = 1.17$) and of two target polymers, PEO $_{43}$ -*b*-PDEA $_{20}$ -*b*-PTA $_{20}$ (**polymer 1**, $M_w = 6100$, $M_w/M_n = 1.20$) and PEO $_{43}$ -*b*-PDEA $_{20}$ -*b*-PTA $_{30}$ (**polymer 2**, $M_w = 6900$, $M_w/M_n = 1.25$).

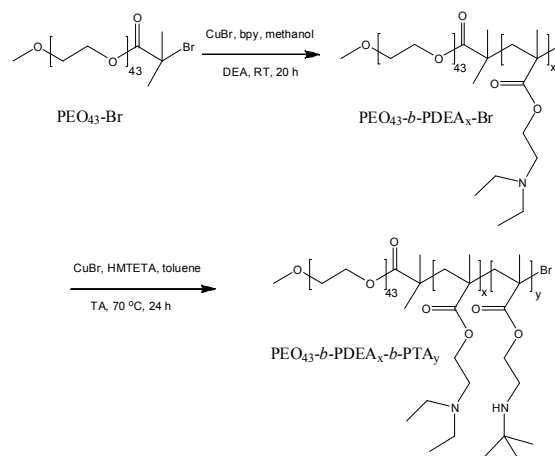


Fig. 1. Synthetic route of PEO-*b*-PDEA-*b*-PTA triblock copolymer.

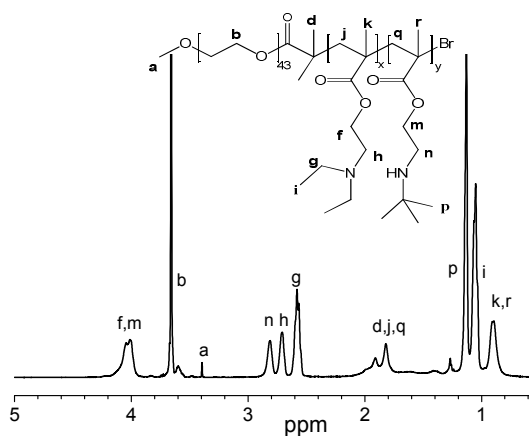


Fig. 2. $^1\text{H NMR}$ spectrum of PEO $_{43}$ -*b*-PDEA $_{20}$ -*b*-PTA $_{20}$ copolymer.

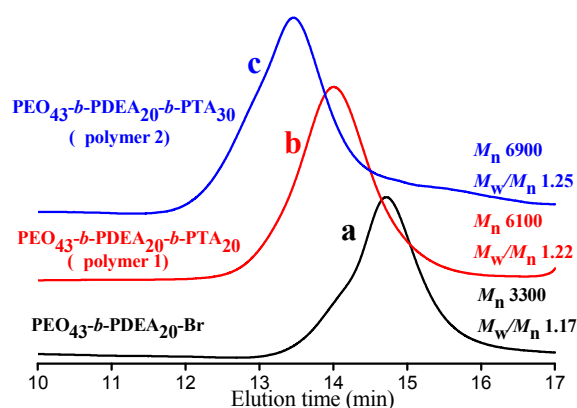


Fig. 3. GPC trace of triblock copolymers in DMF.

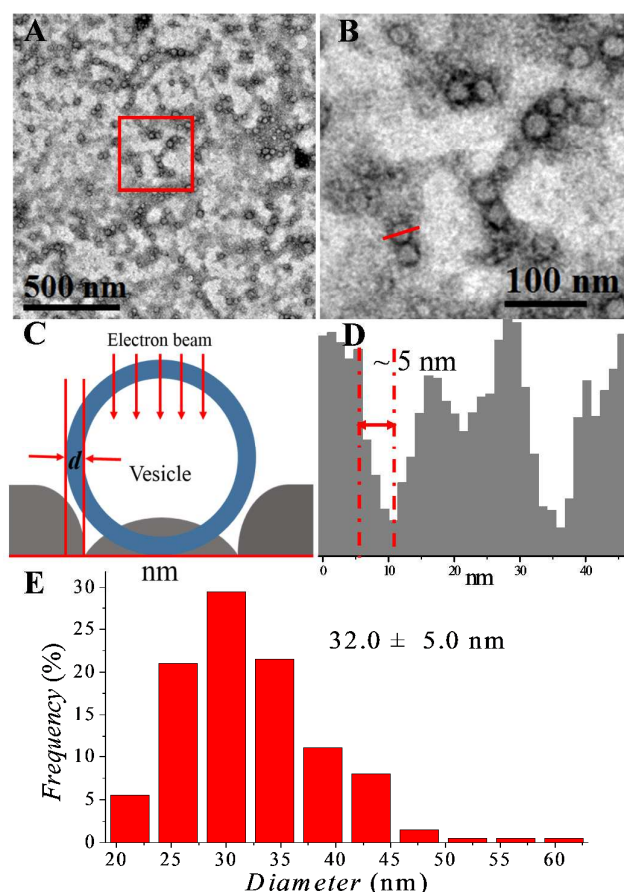


Fig. 4. TEM analysis of polymersomes formed by **polymer 1**. D is the electron transmittance chart related to the red scan-line in B, revealing a 5 nm of membrane thickness of polymersomes at dry state. E reveals a statistical mean diameter of 32 nm calculated by image A.

Preparation of PEO-*b*-PDEA-*b*-PTA polymersome.

The polymersomes were obtained by simply adding NaOH solution (pH 11) into copolymer solution in deionized water at an initial pH of 1. During the process of self-assembly, the

colorless solution turned into bluish when pH reached 7.4, indicating the formation of polymersomes. Considering that PTA is partially hydrophilic and partially hydrophobic in neutral water,³⁴ the hydrophilic PEO and partial PTA chains form the corona while the hydrophobic PDEA and partial PTA chains form the membrane.

The vesicular structure of the polymersomes was confirmed by TEM studies (Fig. 4).³⁵ The mean diameter of polymersomes is 32 nm. According to our recently developed protocol,⁵ the thickness of the membrane is *ca.* 5 nm.

DLS study of polymersomes indicated a mean diameter of 37.9 nm (Fig. 5), which was in accordance with TEM studies (32.0 nm), but with a relatively high PDI of 0.411. This can be explained by TEM image in Fig. 4A that there are different levels of aggregation between polymersomes thus resulting in an uneven diameter distribution.

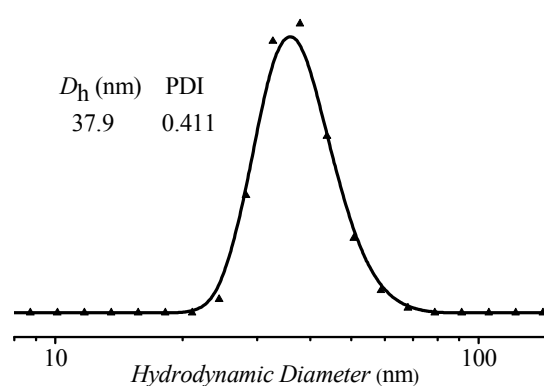


Fig. 5. DLS study of PEO₄₃-*b*-PDEA₂₀-*b*-PTA₂₀ polymersomes in water at 1.0 mg/mL and 25 °C.

Furthermore, the zeta potential values of polymersomes made from PEO₄₃-*b*-PDEA₂₀-*b*-PTA₂₀ and PEO₄₃-*b*-PDEA₂₀-*b*-PTA₃₀ at pH 7.4 are +48.8 mV and +53 mV, respectively, indicating that the polymersome possess positive charges on the surface.

Antibacterial activities of PEO-*b*-PDEA-*b*-PTA polymersome.

Compared with individual polymer chains, the polymersome is more likely to exhibit excellent antibacterial ability because the increased local mass and positive charges result in a more efficient interaction with the cell membrane. The antibacterial efficiency of the polymersome was evaluated by the Minimal Inhibitory Concentration (MIC), which is defined as the minimum concentration of an antimicrobial agent at which no visible growth of microbes is observed. The MICs of the polymersome were measured using a broth micro-dilution method and the value was taken at the concentration where no growth was observed in the phase of microbes through a visible spectrophotometer. As shown in Table 1, the MIC values of the polymersome formed from **polymer 1** (defined as **polymersome 1**) against *E. coli* and *S. aureus* (which are selected to represent Gram-negative and Gram-positive

bacteria) after 24 h are 0.300 and 0.600 mM, respectively. In contrast, those of the polymersomes formed from **polymer 2** (defined as **polymersome 2**) are 0.150 and 0.600 mM.

Table 1. MIC values of **polymersome 1** and **polymersome 2**. "+" represents the growth of the bacteria and "-" represents no growth.

C/mM	<i>E. coli</i>		<i>S. aureus</i>	
	1	2	1	2
1.20	-	-	-	-
0.600	-	-	-	-
0.300	-	-	+	+
0.150	+	-	+	+
0.075	+	+	+	+
0.038	+	+	+	+

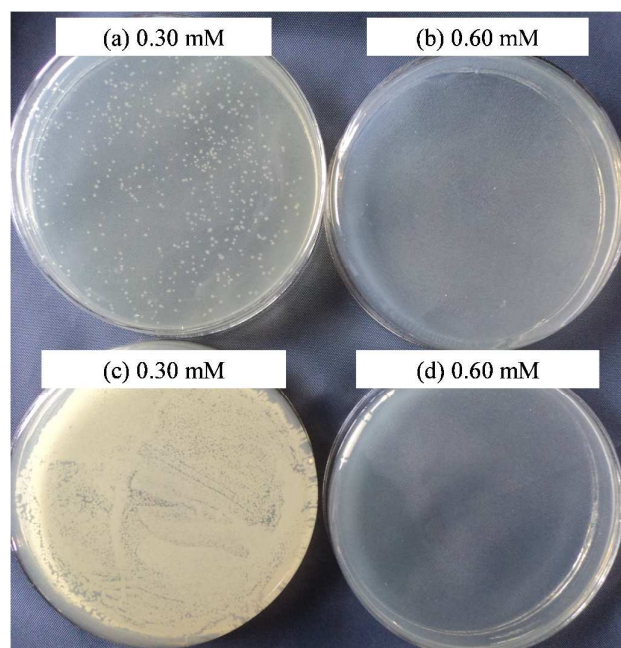


Fig. 6. Digital photographs of LB agar with **polymersome 1** solutions at different concentrations: (a, b) against *E. coli*; (c, d) against *S. aureus*. The visible white dot is the bacterial colonies.

The investigation revealed that the both kinds of polymersomes showed good antibacterial activities. Furthermore, the lower MIC value of **polymersome 2** against *E. coli* indicates its higher antibacterial efficiency, as a result of the higher content of PTA segments in the polymersome, and consequently higher density of positive charge on the surface.

To further investigate the antibacterial ability of both polymersomes, a colony formation assay is performed to measure the Minimal Bactericidal Concentration (MBC), which is defined as the minimum concentration of an antimicrobial

agent at which 99.9 % microbes are killed. The value was taken at the concentration where no living microbes were observed in the glass sheet and the number of Colony-Forming Units (CFUs) was counted to quantify the antibacterial efficiency. The MBCs of **polymersome 1** against *E. coli* and *S. aureus* after 24 h are 0.60 and 0.60 mM, respectively (Fig. 6). Sporadic (Fig. 6a) or dense (Fig. 6c) bacterial colonies were observed in the treated sample with 0.300 mM of polymersomes while no visible bacterial colonies remain in the treated sample with 0.600 mM of polymersomes (Fig. 6c, d). As for **polymersome 2**, the MBCs against *E. coli* and *S. aureus* after 24 h are 0.30 and 1.20 mM, respectively (Fig. 7). The investigation of MBC further confirms that polymersomes formed from two copolymers exhibit good antibacterial ability.

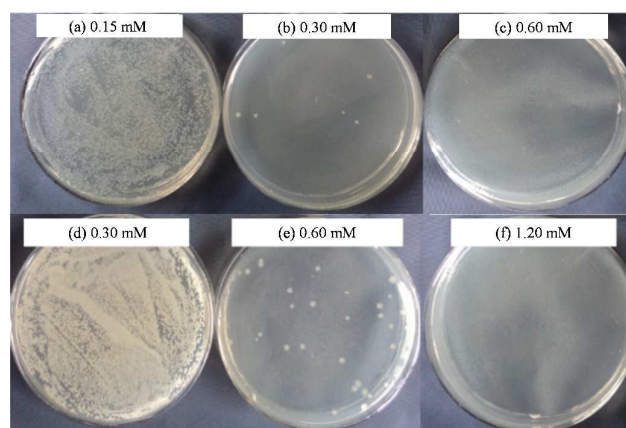


Fig. 7. Digital photographs of LB agar with **polymersome 2** solutions at different concentrations: (a, b, c) against *E. coli*; (d, e, f) against *S. aureus*. The visible white dot is the bacterial colonies.

Conclusions

In summary, we have successfully synthesized novel pH-sensitive PEO-*b*-PDEA-*b*-PTA triblock copolymers *via* ATRP that can self-assemble into antibacterial polymersomes by simple dissolution in acid water and adjusting the pH to 7.4, which greatly simplifies the self-assembly process. The pH-sensitive PDEA chains form the polymersome membrane, which may be further functionalized for further "upgrading". The biocompatible PEO and antibacterial PTA form the polymersome corona. These stable polymersomes showed good antibacterial properties against both Gram-positive and Gram-negative bacteria with low MICs and MBCs, suggesting a wide range of antibacterial applications. This protocol also provides us with a new insight for preparing antibacterial polymeric formulations which may be facilely sprayed on places which are susceptible to bacterial attack such as hospitals for long-term antibacterial applications.

Acknowledgements

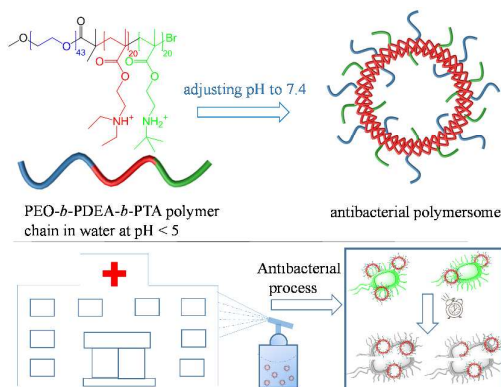
This work is supported by NSFC (21174107 and 21374080), Shanghai 1000 Plan, and Eastern Scholar Professorship.

Notes and references

- X. L. Hu, G. H. Liu, Y. Li, X. R. Wang and S. Y. Liu, *J. Am. Chem. Soc.*, 2015, 137, 362-368.
- J. Z. Du and S. P. Armes, *Soft Matter*, 2010, 6, 4851-4857.
- Q. T. Huang, B. Yang, H. H. Liu, Y. L. Zhao and J. Z. Du, *Polym. Chem.*, 2015, 6, 886-890.
- G. H. Liu, X. R. Wang, J. M. Hu, G. Y. Zhang and S. Y. Liu, *J. Am. Chem. Soc.*, 2014, 136, 7492-7497.
- Y. Q. Zhu, F. Y. K. Wang, C. Zhang and J. Z. Du, *ACS Nano*, 2014, 8, 6644-6654.
- M. Z. Wang, C. C. Zhou, J. Chen, Y. F. Xiao and J. Z. Du, *Bioconjugate Chem.*, 2015, 26, 725-734.
- C.-C. Liu, A. Ramírez-Hernández, E. Han, G. S. W. Craig, Y. Tada, H. Yoshida, H. Kang, S. Ji, P. Gopalan, J. J. de Pablo and P. F. Nealey, *Macromolecules*, 2013, 46, 1415-1424.
- H. Kong and J. Jang, *Langmuir*, 2008, 24, 2051-2056.
- S. K. Mehta, S. Chaudhary, S. Kumar and S. Singh, *J. Nanopart. Res.*, 2010, 12, 1697-1709.
- T. F. Zhu, I. Budin and J. W. Szostak, *Methods Enzymol.*, 2013, 533, 267-274.
- J. Z. Du and S. P. Armes, *Langmuir*, 2009, 25, 9564-9570.
- S. Zheng, Y. Zheng, R. L. Beissinger and R. Fresco, *Biochim. Biophys. Acta, Biomembr.*, 1994, 1196, 123-130.
- J. Z. Du, Y. Q. Tang, A. L. Lewis and S. P. Armes, *J. Am. Chem. Soc.*, 2005, 127, 17982-17983.
- J. Z. Du, L. Fan and Q. M. Liu, *Macromolecules*, 2012, 45, 8275-8283.
- Y. T. Li, B. S. Lokitz and C. L. McCormick, *Angew. Chem., Int. Ed.*, 2006, 45, 5792-5795.
- Y. Morishima, *Angew. Chem., Int. Ed.*, 2007, 46, 1370-1372.
- T.-H. Kim, Y.-S. Han, B.-S. Seong and K.-P. Hong, *Soft Matter*, 2011, 7, 10070-10075.
- J. C. Arthur, E. Perez-Chanona, M. Mühlbauer, S. Tomkovich, J. M. Uronis, T.-J. Fan, B. J. Campbell, T. Abujamel, B. Dogan, A. B. Rogers, J. M. Rhodes, A. Stintzi, K. W. Simpson, J. J. Hansen, T. O. Keku, A. A. Fodor and C. Jobin, *Science*, 2012, 338, 120-123.
- C. C. Zhou, M. Z. Wang, K. D. Zou, J. Chen, Y. Q. Zhu and J. Z. Du, *ACS Macro Lett.*, 2013, 2, 1021-1025.
- M. Z. Wang and J. Z. Du, *Acta Polym. Sin.*, 2014, 0, 1183-1194.
- J. Song, H. Kong and J. Jang, *Chem. Commun.*, 2009, DOI: 10.1039/b908060k, 5418.
- S. Liu and G. Sun, *Ind. Eng. Chem. Res.*, 2006, 45, 6477-6482.
- G. Seyfriedsberger, K. Rametsteiner and W. Kern, *Eur. Polym. J.*, 2006, 42, 3383-3389.
- K. D. Zou, Q. M. Liu, J. Chen and J. Z. Du, *Polym. Chem.*, 2014, 5, 405-411.
- M. B. Harney, R. R. Pant, P. A. Fulmer and J. H. Wynne, *ACS Appl. Mater. Interfaces*, 2009, 1, 39-41.
- E.-R. Kenawy, F. I. Abdel-Hay, A. E.-R. R. El-Shanshoury and M. H. El-Newehy, *J. Polym. Sci., Part A: Polym. Chem.*, 2002, 40, 2384-2393.
- C. M. Moore, S. Hackman, T. Brennan and S. D. Minteer, *J. Membr. Sci.*, 2005, 254, 63-70.
- M. Ignatova, S. Voccia, B. Gilbert, N. Markova, D. Cossement, R. Gouttebaron, R. Jérôme and C. Jérôme, *Langmuir*, 2005, 22, 255-262.
- A. J. Morse, D. Dupin, K. L. Thompson, S. P. Armes, K. Ouzineb, P. Mills and R. Swart, *Langmuir*, 2012, 28, 11742-11753.
- S. Lenoir, C. Pagnoulle, M. Galleni, P. Compère, R. Jérôme and C. Detrembleur, *Biomacromolecules*, 2006, 7, 2291-2296.
- Q. R. Geng, J. G. Xiao, B. Yang, T. Wang and J. Z. Du, *ACS Macro Lett.*, 2015, 4, 511-515.
- J. Chen, F. Y. K. Wang, Q. M. Liu and J. Z. Du, *Chem. Commun.*, 2014, 50, 14482-14493.
- W. Z. Yuan, J. R. Wei, H. Lu, L. Fan and J. Z. Du, *Chem. Commun.*, 2012, 48, 6857-6859.
- H. S. Zhu, Q. R. Geng, W. Q. Chen, Y. Q. Zhu, J. Chen and J. Z. Du, *J. Mater. Chem. B*, 2013, 1, 5496-5504.
- J. Z. Du and Y. M. Chen, *Macromolecules*, 2004, 37, 5710-5716.

For Table of Contents Graphic only:

We report the facile synthesis of antibacterial polymersomes in pure water, which show good antibacterial activities against both Gram-positive and Gram-negative bacteria and can be sprayed in places which are susceptible to bacterial attack for long-term effective antibacterial applications.



Electronic Supplementary Information for

Preparation of polymersomes in pure water for facile antibacterial applications

Tao Wang,^a Jinhui Jiang,^a Yufen Xiao,^a Yijie Zou,^a Jingyi Gao,^a and Jianzhong Du^{a,b,*}

^a Key Laboratory of Advanced Civil Engineering Materials of Ministry of Education, School of Materials Science and Engineering, Tongji University, 4800 Caoan Road, Shanghai 201804, China.

^b Shanghai 10th People's Hospital, Tongji University School of Medicine, 301 Middle Yanchang Road, Shanghai 200072, China.

Email: jzdu@tongji.edu.cn; Fax: +86-21-6958 4723; Tel: +86-21-6958 0239

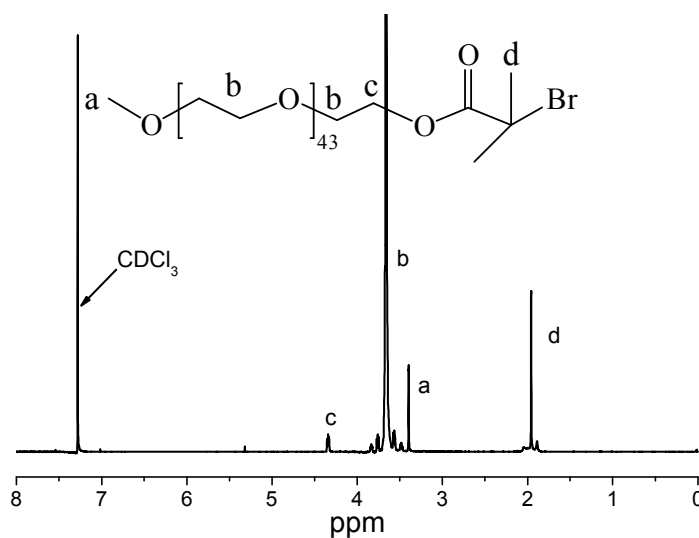


Fig. S1. ¹H NMR spectrum of PEO₄₃-Br. The easterification efficiency is over 95%, which was calculated by comparing peak d with peak b.

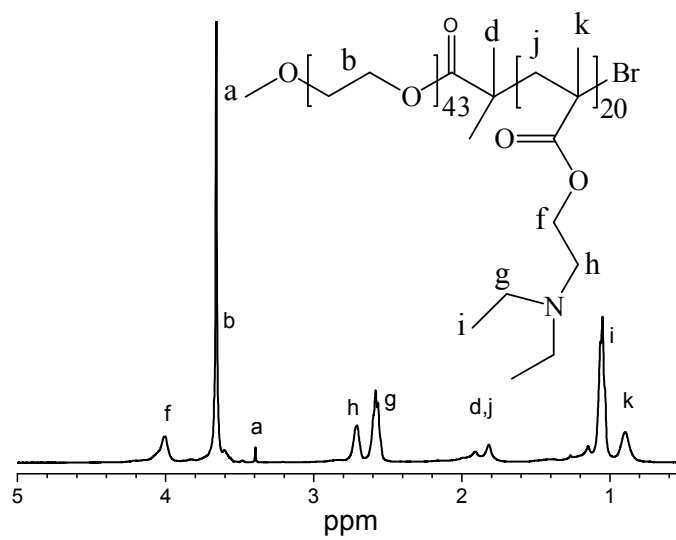


Fig. S2. ¹H NMR spectra of PEO₄₃-b-PDEA₂₀-Br. Peaks a, b and d are assigned to the protons from PEO-Br. Peaks f to k are attributed to the protons of PDEA.