Preparation of Poly(MTZ)$_n$−(DMAEMA)$_m$ Micelles and Study on Their Antibacterial Property

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ABSTRACT: Bacterial infections are the most common type of clinical infection. The abuse of clinical antibiotics has led to the frequent appearance of drug-resistant strains and even some super bacteria. In this study, we synthesized Poly(MTZ)$_n$−(DMAEMA)$_m$ polymer micelles with cations on the surface. The synthesis of this novel polymer comes in two steps. First, Poly(MTZ)$_n$ was synthesized with metronidazole (MTZ) referred as the macro-molecular chain transfer agent and v-501 as the initiator for initiating the polymerization of 4-cyanopentanoic acid dithiobenzoate. Then, novel polymer micelles were synthesized with Poly(MTZ)$_n$ referred as the macromolecular chain transfer agent and v-501 as the initiator for initiating the polymerization of the monomer 2-(dimethylamino) ethyl methacrylate, which could adsorb to the negatively charged bacterial surface via electrostatic interaction and enhance bactericidal activity. Scanning electron microscopy showed that the micelles could be accurately targeted to the surface of bacteria, and the zone of inhibition assay confirmed that the micelles could enhance the sensitivity of bacteria to drugs. Hence, Poly(MTZ)$_n$−(DMAEMA)$_m$ polymer micelles will have potential use for the clinical treatment of anaerobic infections in the future.

1. INTRODUCTION

Bacterial infections are the most common type of clinical infection. Anaerobic infections account for more than 70% of oral, thoracic, abdominal, and pelvic infections, which have become a major problem in clinical bacterial infection treatment.$^{1,2}$ Metronidazole (MTZ), a kind of antibiotic, is the first generation of S′-nitroimidazole, which has been used in clinical medicine.$^3$ It is highly active against Gram-negative anaerobic bacteria, such as Bacteroides fragilis (B. fragilis), and Gram-positive anaerobic bacteria, such as Clostridium difficile.$^4,5$ The antibacterial mechanism of anaerobic bacteria indicates that the nitro group is reduced to an amino group under an anaerobic environment, or the formation of free radicals breaks the helical structure of the bacterial chromosome, blocking its transcriptional replication.$^6$ In general traditional antibiotic treatment methods, systemic medicine is inefficient because of insufficient local effective drug concentration, which is time-consuming, and the drug may be gradually replaced by local medicine because of adverse reactions to nontreatment sites.$^7$ However, local drug treatment is still limited by certain characteristics of the drug, such as a high degradation rate, and it is easy to induce bacterial resistance. Therefore, the drug is loaded and released locally to produce a strong and lasting antibacterial effect that has become the focus of the following research.

Biosynthesis of polymer micelles is a fast-growing research in the field of polymer materials$^{8–11}$ because of their unique biological, optical, and physiochemical properties, which have been extensively studied in the arena of biomedicine.$^{12–15}$ Especially, they have been explored as one of the main nanocarriers for cancer nanomedicine, which showed the benefit of drug solubilization, aiming to enhance the permeability and retention effect by delivering drugs to tumors.$^{16–18}$ We have designed a kind of MTZ-based polypropdrug [Poly(MTZ)$_n$] as a hydrophobic core of hypoxia-responsive nanoparticle carrier. This nanoparticle has been shown to elevate the targeting ability of gliomas and reduce toxic and side effects on normal tissues in our previous study.$^{19,20}$ Thus, biodegradable nitroimidazole polymers were synthesized as the hydrophobic cores of polymer micelles, which could enhance the loading efficiency and antibacterial effect in this study.

Received: June 11, 2020
Accepted: August 14, 2020
Published: September 1, 2020
In this study, we designed Poly(MTZ)\textsuperscript{n−(DMAEMA)}\textsubscript{m} polymer micelles with cations on the surface with Poly(MTZ)\textsubscript{n} as the macromolecular chain transfer agent and v-501 as the initiator for initiating the polymerization of the monomer 2-\(\text{dimethylamino}\) ethyl methacrylate (DMAEMA), which could adsorb to the negatively charged bacterial surface via electrostatic interaction and enhance bactericidal activity\textsuperscript{21−23} that is demonstrated to effectively kill anaerobic bacteria under anoxic conditions. The Poly(MTZ)\textsuperscript{n−(DMAEMA)}\textsubscript{m} micelles comprise three distinct functional components: (1) DMAEMA can be connected with Poly(MTZ)\textsubscript{n} to form a polymer with cations on its surface, thus enabling this polymer with the ability to target the negatively charged bacterial surface; (2) nitro groups of the Poly(MTZ)\textsubscript{n} core are converted into amino groups under an anaerobic environment to break the helical structure of the bacterial chromosome; (3) the hydrophobic Poly(MTZ)\textsubscript{n} core could enhance the loading efficiency. These features make Poly(MTZ)\textsuperscript{n−(DMAEMA)}\textsubscript{m} micelles accurately located on the surface of bacteria, mentioning the local antibiotic concentration in the injection site to reduce bacterial resistance. The zone of inhibition assay proved that our polymer micelles were highly sensitive to bacteria and could inhibit the bacterial proliferation effectively under the same conditions as free-MTZ (Scheme 1).

2. RESULTS AND DISCUSSION

2.1. Synthesis of Poly(MTZ)−(DMAEMA). In order to synthesize Poly(MTZ)\textsuperscript{n−(DMAEMA)}\textsubscript{m}, Poly(MTZ-MA) was first synthesized and then reacted with DMAEMA to form the final product. First, we combine MTZ monomer with methacrylic acid (MA) to produce MTZ-MA as a structural unit, which is shown in Figure 1A. The \(\text{H}\) NMR nuclear magnetic resonance (NMR) results demonstrate that MTZ-MA was successfully synthesized (Figure 1B). Next, we combine MTZ-MA with 4-cyanopentanoic acid dithiobenzoate (CPADB) to form Poly(MTZ)\textsubscript{n} with different degrees of polymerization. Finally, as shown in Figure 2A, Poly(MTZ)\textsuperscript{n−(DMAE-MEA)}\textsubscript{m} was synthesized via reaction of Poly(MTZ)\textsubscript{n} and DMAEMA. To compare the bacteriostatic effect of the polymer with different degrees of polymerization, we synthesized a total of three polymers, Poly(MTZ)\textsubscript{20−(DMAEMA)}\textsubscript{60}, Poly(MTZ)\textsubscript{67−(DMAEMA)}\textsubscript{60}, and Poly(MTZ)\textsubscript{34−(DMAEMA)}\textsubscript{69}. All these polymers were characterized by \(\text{H}\) NMR and gel permeation chromatography (GPC) (Figure 2B, Table 1).\textsuperscript{24} Taking Poly(MTZ)\textsubscript{30−(DMAEMA)}\textsubscript{60} as an example, in the \(\text{H}\) NMR spectrum, the four characteristic peaks [\(\delta\) 2.67 (a), \(\delta\) 8.09 (b), \(\delta\) 4.75 (c), and \(\delta\) 4.23 (d)] on Poly(MTZ)\textsubscript{20} could be clearly seen in Figure 2B(a). The peaks of the chemical shifts [\(\delta\) 4.62 (e), \(\delta\) 2.56 (f), and \(\delta\) 2.34 (g); the red arrows indicate the characteristic peaks of Poly(MTZ)\textsubscript{n−(DMAEMA)}\textsubscript{m}] are characteristic peaks of Poly(MTZ)\textsubscript{20−(DMAEMA)}\textsubscript{60} from Figure 2B(b). By comparing the characteristic peak of Poly(MTZ)\textsubscript{20} with the characteristic peak of Poly(MTZ)\textsubscript{20−(DMAEMA)}\textsubscript{60}, it can be seen that the polymer conjugate Poly(MTZ)\textsubscript{20−(DMAEMA)}\textsubscript{60} was synthesized successfully. The other two groups [Poly(MTZ)\textsubscript{67−(DMAEMA)}\textsubscript{60} and Poly(MTZ)\textsubscript{34−(DMAEMA)}\textsubscript{69}] could also prove the successful combination of Poly(MTZ)\textsubscript{n} and DMAEMA through \(\text{H}\) NMR (Figure 2B(c−f), respectively; the red arrows indicate the characteristic peaks of Poly(MTZ)\textsubscript{n−(DMAEMA)}\textsubscript{m}]. Meanwhile, GPC data (Table 1) showed that different degrees of polymerization of Poly(MTZ)\textsubscript{n} and

![Scheme 1. Schematic of Poly(MTZ)\textsubscript{n−(DMAEMA)}\textsubscript{m} Adsorption to the Negatively Charged Bacterial Surface via Electrostatic Interaction](image)

![Figure 1. (A) Chemical structure and synthesis route of MTZ-MA. (B) \(\text{H}\) NMR spectrum (300 MHz, DMSO) of MTZ-MA.](image)
Poly(MTZ)$_n$−(DMAEMA)$_m$ had a single peak, and the molecular weight of Poly(MTZ)$_n$−(DMAEMA)$_m$ was significantly higher. Through the above data, we have not only proved the successful conjugate of the polymer but also proved that the purity of our polymer is high and that there is no other substance. In addition, in order to verify the inhibitory effect of MTZ on anaerobic bacteria in the later stage, we set Im as the experimental control group. Poly(Im)$_n$−(DMAEMA)$_m$ was synthesized as the control in order to validate the bacteriostasis of Poly(MTZ)$_n$−(DMAEMA)$_m$. The chemical structure of Im-

| copolymer                  | Mn (GPC) | PDI (GPC) |
|----------------------------|----------|-----------|
| Poly(MTZ)$_{20}$           | 5200     | 1.13      |
| Poly(MTZ)$_{20}$−(DMAEMA)$_{60}$ | 11,200   | 1.41      |
| Poly(MTZ)$_{34}$           | 6400     | 1.13      |
| Poly(MTZ)$_{34}$−(DMAEMA)$_{69}$ | 14,900   | 1.41      |
| Poly(MTZ)$_{67}$           | 8300     | 1.14      |
| Poly(MTZ)$_{67}$−(DMAEMA)$_{60}$ | 14,900   | 1.41      |

The red arrows indicate the characteristic peaks of Poly(MTZ)$_n$−(DMAEMA)$_m$. 

Table 1. Molecular Characteristics of Poly(MTZ)$_n$ and Poly(MTZ)$_n$−(DMAEMA)$_m$
MA and the final product Poly(Im)_n-(DMAEMA)_m are shown in Figure 3A and their characteristics were confirmed by 1H NMR spectroscopy with characteristic peaks and integration values (Figure 3B).

2.2. Preparation and Characterization of Poly-(MTZ)_n-(DMAEMA)_m Micelles. After the successful synthesis of Poly(MTZ)_n-(DMAEMA)_m, Poly(MTZ)_n-(DMAEMA)_m micelles were prepared and characterized by dynamic light scattering (DLS) and transmission electron microscopy (TEM). Synthesized Poly(MTZ)_n-(DMAEMA)_m was directly dissolved in N,N-dimethylformamide solution and slowly dripped into distilled water to form a nanomicelle, which was convenient for bacteriostatic treatment. The nanosize of Poly(MTZ)_n-(DMAEMA)_m micelles was measured by DLS.25 As can be seen from Figure 4A and Table 2, the average hydrodynamic diameters of Poly(MTZ)_20-(DMAEMA)_60 Poly(MTZ)_60-(DMAEMA)_60 and Poly(MTZ)_34-(DMAEMA)_69 were 128.2 ± 3.7, 112.1 ± 1.9, and 98.2 ± 2.7 nm and the polydispersity indices (PDIs) were 1.14 ± 0.3, 1.18 ± 0.1, and 1.13 ± 0.2, respectively. As shown in Figure 4B–D, using TEM, it can be seen that Poly(MTZ)_34-(DMAEMA)_69 Poly(MTZ)_20-(DMAEMA)_60 and Poly(MTZ)_34-(DMAEMA)_69 micelles were spherical micelles with monodisperse and unimodal size distribution.26,27 Under anoxic conditions, the micelles with hydrophobic Poly(MTZ)_n as the core would crack,28 so the hydrodynamic diameter will become scattered from Figure 4E. Meanwhile, the average zeta potentials were 34.8 ± 0.7, 35.3 ± 0.5, and 33.1 ± 0.7 mV (Figure 4F). The polymer micelles with positive charges could adsorb to the negatively charged bacterial surface via electrostatic interaction and enhance bactericidal activity.

2.3. Poly(MTZ)_n-(DMAEMA)_m Micelles Could Adsorb to Bacteria. The morphology of micelle-coated B. fragilis was first observed by scanning electron microscopy (SEM) to obtain direct insight on the interaction between B. fragilis and micelles. As shown in Figure 5A, the uncoated naked B. fragilis evinced a typical rod shape with a smooth membrane surface, whereas polyplex micelles were clearly shown sticking on the B. fragilis surface in the case of micelle-coated B. fragilis. Because of the electronegative nature of bacterial cell walls, the positively charged micelles can be self-assembled onto the B. fragilis surface via electrostatic interaction, which results in the formation of a dense coating layer over the rod-shaped B. fragilis. Meanwhile, the strong electrostatic and hydrophobic interactions between polyplex micelles and bacteria may damage the cell wall of bacteria.28 We can see from Figure 5B that after 12 h of drug action, the bacterial damage of the synthetic polymer micelle experimental group was the most severe; also a large number of bacteria were seen to be lysed. However, only a small amount of bacteria cracking occurs in the free-MTZ group under the same conditions. The above data indicate that polymer micelles can increase the sensitivity of bacteria to drugs.

2.4. Minimum Inhibitory Concentration. Furthermore to study if the bacteriostatic effect of Poly(MTZ)_n-(DMAEMA)_m is better than that of the monomer MTZ, we
explored the minimal inhibitory concentrations (MICs) of the polymer micelles.\textsuperscript{29,30} We can see from Table 3 that the MIC of the monomer MTZ is 5 mg/mL and that of the overall polymer micelles is 2.5 mg/mL. The above data reflect that the micelles are more sensitive to the killing effect of anaerobic bacteria.

### 2.5. Zone of Inhibition Assay Shows Antibacterial Activity

The above studies have shown that the antibacterial effect of polymer micelles is significantly higher than that of monomer MTZ. We use the zone of inhibition assay to verify the antibacterial activity\textsuperscript{31} using a filter paper with a diameter of 8 mm, loaded with polymers of the same mass concentration, incubated on anaerobic agar medium plates, and incubated anaerobically at 37 °C for 48 h using a 2.5 L round-bottom vertical anaerobic culture bag. As shown in Figure 6A, we can see that the zone of inhibition of free-MTZ is significantly smaller than that of our polymer micelles, and neither DMAEMA nor phosphate-buffered saline (PBS) groups showed zone of inhibition, proving that DMAEMA has no effect on bacteria. The results showed that the polymer micelle group and free-MTZ group had significant statistical significance (Figure 6D, **\( p = 0.0062 \)). Next, we began to study the antibacterial effects of micelles with different degrees of polymerization at the same concentration. We set Poly(Im)\textsubscript{n}−(DMAEMA)\textsubscript{m} as the experimental control group in order to verify the inhibitory effect of Poly(MTZ)\textsubscript{n}−(DMAEMA)\textsubscript{m} on anaerobic bacteria. We also used the zone of inhibition assay to verify the antibacterial activity using a filter paper with a diameter of 8 mm, loaded with polymers of the same mass concentration, incubated on anaerobic agar medium plates, and incubated anaerobically at 37 °C for 48 h using a 2.5 L round-bottom vertical anaerobic culture bag. As shown in Figure 6B, we can see that the antibacterial effect of the Poly(MTZ-MA)\textsubscript{34}−(DMAEMA)\textsubscript{69} micellar group is significantly higher than those of other groups, and this group was statistically significant with **\( p = 0.0062 \). 

### Table 2. Characterization of Poly(Im)\textsubscript{n}−(DMAEMA)\textsubscript{m} and Poly(MTZ)\textsubscript{n}−(DMAEMA)\textsubscript{m} Micelles

| copolymer               | size (nm)       | PDI     | zeta potential (mV) |
|-------------------------|-----------------|---------|---------------------|
| Poly(Im)\textsubscript{34}−(DMAEMA)\textsubscript{69} | 136.4 ± 4.2     | 0.26 ± 0.06 | 38.3 ± 0.3          |
| Poly(MTZ)\textsubscript{20}−(DMAEMA)\textsubscript{60} | 128.2 ± 3.7     | 0.17 ± 0.02 | 34.8 ± 0.7          |
| Poly(MTZ)\textsubscript{67}−(DMAEMA)\textsubscript{60} | 112.1 ± 1.9     | 0.19 ± 0.03 | 35.3 ± 0.5          |
| Poly(MTZ)\textsubscript{34}−(DMAEMA)\textsubscript{69} | 98.2 ± 2.7      | 0.15 ± 0.04 | 33.1 ± 0.7          |
3. CONCLUSIONS

In summary, we constructed a Poly(MTZ)\textsubscript{10}−(DMAEMA)\textsubscript{m} polymer with cations on the surface for adsorbing to the negatively charged bacterial surface via electrostatic interaction. It also has a significant targeting effect which was demonstrated by SEM. In the zone of inhibition assay, Poly(MTZ)\textsubscript{10}−(DMAEMA)\textsubscript{m} significantly inhibited the colony of bacterial growth compared with free-MTZ. Meanwhile, the bacteriostatic effect of Poly(MTZ)\textsubscript{34}−(DMAEMA)\textsubscript{69} polymer micelles was the best under different degrees of polymerization. This finding suggested that the Poly(MTZ)\textsubscript{10}−(DMAEMA)\textsubscript{m} micelles will have potential use for clinical treatment of anaerobic infections in the future.

4. EXPERIMENTAL SECTION

4.1. Materials. DMAEMA (Aldrich, 98%) was from Sigma-Aldrich (St. Louis, Missouri, USA) and was purified with an alumina column. 4,4′-Azo (4-cyanovaleric acid) (v-501, Aldrich, 99%, produced by J&K Technology Co., Ltd., Beijing, China) was purified by methanol precipitation and vacuum dried at 25 °C overnight. B. fragilis and anaerobic agar medium plate were purchased from Nanjing Yasong Biotechnology Co., Ltd.

4.2. Preparation of MTZ-MA. MTZ-MA has been synthesized successfully in our previous research.\textsuperscript{19} MTZ (8.55 g, 50 mmol), MA (6.45 g, 75 mmol), and 4-dimethylaminopyridine (3.05 g, 25 mmol) were dissolved in dry dichloromethane (DCM). The mixture was stirred under nitrogen conditions with dicyclohexylcarbodiimide (20.6 g, 100 mmol, dissolved in DCM) being added dropwise. Under 30 °C conditions, the mixture was stirred overnight. The product was then purified by flash column chromatography on silica gel. Eluting with a mixed solvent of PE/EA (v/v = 1/1) afforded MTZ-MA (10.9 g, yield 91.5%) as a white solid.

4.3. Preparation of Poly(MTZ-MA). Poly(MTZ-MA) has been synthesized successfully in our previous research.\textsuperscript{19} MTZ-MA (718 mg, 300 mmol), v-501 (4.8 mg, 30 mmol), CPADB (16.8 mg, 60 mmol), and dimethyl sulfoxide (DMSO) (1 mL) were added to the tube. The solution was deoxygenized with three standard freeze−pump−thaw cycles. Then, the reaction tube was put into a preset oil bath at 70 °C. After keeping the reaction tube at 70 °C for a predetermined time, it was put in a cooling water bath. The polymer obtained after adding methanol was crude oil, which was vacuum dried at 25 °C for 24 h. MWNMR = 5300 Da, PDI = 1.12, polymerization time = 8 h; MWNMR = 6400 Da, PDI = 1.14, polymerization time = 12 h; MWNMR = 7700 Da, PDI = 1.13, polymerization time = 16 h.

4.4. Preparation of Poly(MTZ)\textsubscript{10}−(DMAEMA)\textsubscript{m}. Poly(MTZ-MA) (600 mg, 0.1 mmol), v-501 (11.3 mg, 0.04 mmol), DMAEMA (8.44 mL, 50 mmol), and DMSO (8.0 mL) were added to tube. The solution was deoxygenized with three standard freeze−pump−thaw cycles. Then, the reaction tube was put into a preset oil bath at 70 °C. After the reaction tube was maintained at 70 °C for a predetermined time, it was placed in a cooling water bath. The polymer was obtained after cooling hexane to a yellow solid.

4.5. Preparation of Im-MA. According to the study of Patrickios, Im-MA was successfully synthesized.\textsuperscript{32} Imidazole (Im, 26 g, 0.38 mol) and ethylene carbonate (52 g, 0.59 mol) were transferred to a round-bottom flask filled with toluene.
and refluxed (110 °C) for 6 h. Im reacts with methacryloyl chloride in the presence of Et$_3$N in CHCl$_3$.

4.6. Preparation of Poly(Im)$_n$. According to the study of Patrickios, Poly(Im)$_n$ was successfully synthesized. The solution of Im (1.00 g, 5.55 mmol), CDP chain transfer agent (22.4 mg, 5.55 $\times$ 10$^{-3}$ mol), and v-501 free radical initiator (15.6 mg, 5.55 $\times$ 10$^{-5}$ mol) in 5.54 mL of glacial acetic acid (5.81 g, 96.8 mmol) were transferred to a round-bottom flask equipped with a glass valve and a magnetic stirring bar.

4.7. Preparation of Poly(Im)$_n$–(DMAEMA)$_m$. Poly(Im-MA), v-501, DMAEMA, and DMSO were added to tube. The solution was deoxidized with three standard freeze–pump–thaw cycles. Then, the reaction tube was put into a preset oil bath at 70 °C. After the reaction tube was maintained at 70 °C for a predetermined time, it was placed in a cooling water bath. The polymer was obtained after cooling hexane to a yellow solid.

4.8. Anaerobic Culture. Firstly, 58.4 g of the anaerobic agar was weighed, dissolved in 1000 mL of distilled water, dispensed, and autoclaved at 121 °C for 15 min. Then, the strain was inoculated on the anaerobic agar medium plate and anaerobically incubated at 37 °C for 48 h under anaerobic conditions. The anaerobic bag was equipped with a 2.5 L anaerobic gas-generating bag. This gas-generating bag could create an anaerobic environment. At the same time, there is an oxygen indicator to observe whether there was oxygen in the anaerobic bag. The indicator changing to pink color shows an oxygen-free environment.

4.9. Morphological Characterization of Bacteria. For the morphological characterization of bacteria, at first, the

Figure 6. (A) Inhibition zone images of PBS, DMAEMA (DA), free-MTZ, and Poly(MTZ)$_n$–(DA)$_m$ after 48 h of incubation at 37 °C under anaerobic conditions. (B) Inhibition zone images of micelles with different degrees of polymerization after 48 h of incubation at 37 °C under anaerobic conditions. (C) Antibacterial effects of micelles with different degrees of polymerization. (D) Bar graph showing the diameter of the zone of inhibition (mm) produced by PBS, DA, free-MTZ, and Poly(MTZ)$_n$–(DA)$_m$ against B. fragilis. (E) Bar graph showing the diameter of the zone of inhibition (mm) produced by micelles with different degrees of polymerization. (F). Corresponding statistical results of flat colony counting under directly mixing the micelles with anaerobic bacteria (*$p < 0.05$, **$p < 0.01$).
bacterial suspension of the above treatment group was evaluated for antibacterial performance, and it was fixed overnight with a fixing solution (3% glutaraldehyde solution + 2% polyoxymethylene). Next, it was stained with 1–2% osmium tetroxide for more than 1 h. Then, it was dehydrated by sequential treatments with 50, 70, 80, 90, and ethyl alcohol absolute successively for 15 min. After drying with a critical point dryer, all samples were sprayed with a platinum layer and observed with a scanning electron microscope. SEM images were recorded using a Tencai G2T12 Instrument from FEI Company (U.S.A).

4.10. Antibacterial Activity Tests. Bacterial sensitivity to antibiotics is commonly tested using the inhibition zone experiment, employing antibiotic impregnated disks. A similar test with micelle laden disks was used in this study. A 5 mL suspension of micelles (5 mg/mL) was put for sonication and subsequently filtered through a membrane filter (0.2 µm, 50 mm diameter). The micelle laden filter paper was dried in an oven for 30 min, and small disks of uniform size (8 mm diameter) containing 200 ± 10 µg micelles were punched out. Before using the filter paper, it was put under an ultraviolet lamp overnight. The bacterial suspension (100 µL of 10^8 to 10^6 cfu/mL) was applied uniformly on the surface of a nutrient agar plate before placing the disks on the plate (five per plate). The plate was placed into a 2.5 L round bottom vertical anaerobic culture bag, and then the anaerobic gas generating bag was put into the culture bag to create an anaerobic condition. The oxygen indicator shows pink indicating that the bag is anaerobic. The culture bag was incubated at 37 °C for 24 h, after which the average diameter of the zone of inhibition surrounding the disk was measured with a ruler with up to 1 mm resolution. The mean and standard deviation (SD) reported for each type of polymer micelle and each microbial strain were based on three replicates.

4.11. Statistical Analyses. All data were collected in triplicate, and statistical analysis was performed with SPSS version 25.0. A comparison between the groups was performed using a t-test. p values <0.05 were considered statistically significant. The experimental results are given in the format of mean ± SD in the figures (*p < 0.05, **p < 0.01, and ***p < 0.001).

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Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This work was supported by the National Natural Science Foundation of China (no. 81772665). This work was also financed by the Jiangsu Provincial Commission of Health and Family Planning (no. Q201608). The research was supported by the Six Talents Peak Foundation of Jiangsu Province (no. 2018-WSW-071). This work was supported by a grant from the Postgraduate Research & Practice Innovation Program of Jiangsu Province (no. KYCX19-2236). The research was supported by the Jiangsu Overseas Visiting Scholar Program for University Prominent Young & Middle-aged Teachers and Presidents (2017). This work was also supported by the Xuzhou Science and Technology Plan Project (KC19034).

REFERENCES

(1) Mehmood, M.; Jaffar, N. A.; Nazim, M.; Khasawneh, F. A. Bacteremic skin and soft tissue infection caused by Prevotella loescheii. BMC Infect. Dis. 2014, 14, 162.

(2) Sanchez, D. A.; Martinez, L. R. Underscoring interstrain variability and the impact of growth conditions on associated antimicrobial susceptibilities in preclinical testing of novel antimicrobial drugs. Crit. Rev. Microbiol. 2019, 45, 51–64.

(3) Dione, N.; Khelaf, S.; Lagier, J.-C.; Raoult, D. The aerobic activity of metronidazole against anaerobic bacteria. Int. J. Antimicrob. Agents 2015, 45, 537–540.

(4) Miguel, L.; Molina, I.; Sulleiro, E.; Lópex, I.; Salvador, F.; Molina-Morant, D.; Sánchez-Montalvá, A. Clinical and Epidemiological Characteristics of Patients with Dientamoeba fragilis Infection. Am. J. Trop. Med. Hyg. 2018, 99, 1170–1173.

(5) Menéndez, C.; Fernández-Suarez, J.; Boga Ribeiro, J. A.; Rodríguez-Pérez, M.; Vázquez, F.; Gonzalez-Sotorrios, N.; Rodríguez-Guardado, A. Epidemiological and clinical characteristics of Dientamoeba fragilis infection. Enferm. Infec. Microbiol. Clin. 2019, 37, 290–295.

(6) Cui, S.-F.; Peng, L.-P.; Zhang, H.-Z.; Rasheed, S.; Vijaya Kumar, K.; Zhou, C.-H. Novel hybrids of metronidazole and quinolones: synthesis, bioactive evaluation, cytotoxicity, preliminary antimicrobial mechanism and effect of metal ions on their transportation by human serum albumin. Eur. J. Med. Chem. 2014, 86, 318–334.

(7) Lewis, C. S.; Supronowicz, P. R.; Zhukauskas, R. M.; Gill, E.; Cobb, R. R. Local antibiotic delivery with demineralized bone matrix. Cell TissueBanking 2012, 13, 119–127.

(8) Anzai, R.; Takami, T.; Uchida, Y.; Murakami, Y. Poly(epsilon-caprolactone) (PCL) hybrid sheets containing polymeric micelles:
Effects of inner structures on the material properties of the sheets. *Mater. Sci. Eng.*, C **2017**, *72*, 325−331.

(9) Gao, M.; Yang, Y.; Bergelé, A.; Huang, L.; Zheng, L.; Bowden, T. M. Self-assembly of cholesterol end-capped polymer micelles for controlled drug delivery. *J. Nanobiotechnol.* **2020**, *18*, 13.

(10) Feng, C.; Huang, X. Polymer Brushes: Efficient Synthesis and Applications. *Acc. Chem. Res.* **2018**, *51*, 2314−2323.

(11) Xu, B.; Feng, C.; Hu, J.; Shi, P.; Gu, G.; Wang, L.; Huang, X. Spin-Casting Polymer Brush Films for Stimuli-Responsive and Anti-Fouling Surfaces. *ACS Appl. Mater. Interfaces* **2016**, *8*, 6685−6692.

(12) Dong, H.; Li, Y.; Wen, H.; Shi, D.; Liu, L. Supramolecular polymer micelles self-assembled from alpha-cyclodextrin and PLLA-PCL based copolymers. *J. Controlled Release* **2011**, *152*, e52−e54.

(13) Mai, Y.; Eisenberg, A. Selective localization of preformed nanoparticles in morphologically controllable block copolymer aggregates in solution. *Acc. Chem. Res.* **2012**, *45*, 1657−1666.

(14) Wang, L.; Li, L.; Fan, Y.; Wang, H. Host-guest supramolecular nanosystems for cancer diagnostics and therapeutics. *Adv. Mater.* **2013**, *25*, 3888−3898.

(15) Tao, D.; Feng, C.; Cui, Y.; Yang, X.; Manners, I.; Winnik, M. A.; Huang, X. Monodisperse Fiber-like Micelles of Controlled Length and Composition with an Oligo(p-phenylenevinylene) Core via “Living” Crystallization-Driven Self-Assembly. *J. Am. Chem. Soc.* **2017**, *139*, 7136−7139.

(16) Houdaïdèh, L.; Evans, J. C.; Allen, C. Overcoming the Road Blocks: Advancement of Block Copolymer Micelles for Cancer Therapy in the Clinic. *Mol. Pharm.* **2017**, *14*, 2503−2517.

(17) Blanco, E.; Shen, H.; Ferrari, M. Principles of nanoparticle design for overcoming biological barriers to drug delivery. *Nat. Biotechnol.* **2015**, *33*, 941−951.

(18) Pérez-Herrero, E.; Fernández-Medarde, A. Advanced targeted therapies in cancer: Drug nanocarriers, the future of chemotherapy. *Eur. J. Pharm. Biopharm.* **2015**, *93*, 52−79.

(19) Hua, L.; Wang, Z.; Zhao, L.; Mao, H.; Wang, G.; Zhang, K.; Liu, X.; Wu, D.; Zheng, Y.; Lu, J.; Yu, R.; Liu, H. Hypoxia-responsive lipid-poly-(hypoxic radiosensitized polydrug) nanoparticles for glioma chemo- and radiotherapy. *Theranostics* **2018**, *8*, 5088−5105.

(20) Zong, Z.; Hua, L.; Wang, Z.; Xu, H.; Ye, C.; Pan, B.; Zhao, Z.; Zhang, L.; Lu, J.; Liu, H.; Yu, R. Self-assembled angiopep-2 modified lipid-poly (hypoxic radiosensitized polydrug) nanoparticles delivery TMZ for glioma synergistic TMZ and RT therapy. *Drug Delivery* **2019**, *26*, 34−44.

(21) Hu, Q.; Wu, M.; Fang, C.; Cheng, C.; Zhao, M.; Fang, W.; Chu, P. K.; Ping, Y.; Tang, G. Engineering nanoparticle-coated bacteria as oral DNA vaccines for cancer immunotherapy. *Nano Lett.* **2015**, *15*, 2732−2739.

(22) Zhou, C.; Wang, F.; Chen, H.; Li, M.; Qiao, F.; Liu, Z.; Hou, Y.; Wu, C.; Fan, Y.; Liu, L.; Wang, S.; Wang, Y. Selective Antimicrobial Activities and Action Mechanism of Micelles Self-Assembled by Cationic Oligomeric Surfactants. *ACS Appl. Mater. Interfaces* **2016**, *8*, 4242−4249.

(23) Konnova, S. A.; Lvov, Y. M.; Fakhru’llin, R. F. Nanoshell Assembly for Magnet-Responsive Oil-Degrading Bacteria. *Langmuir* **2016**, *32*, 12552−12558.

(24) Tao, D.; Feng, C.; Lu, Y.; Cui, Y.; Yang, X.; Manners, I.; Winnik, M. A.; Huang, X. Self-Seeding of Block Copolymers with a π-Conjugated Oligo(p-phenylenevinylene) Segment: A Versatile Route toward Monodisperse Fiber-like Nanostructures. *Macromolecules* **2018**, *51*, 2065−2075.

(25) de Kruijf, C. G.; Huppertz, T.; Urban, V. S.; Petukhov, A. V. Casein micelles and their internal structure. *Adv. Colloid Interface Sci.*) **2012**, *171−172*, 36−52.

(26) Sukamporn, P.; Baek, S.; Gritinsapan, W.; Chirachanchai, S.; Nualsanit, T.; Rojanapanthu, P. Self-assembled nanomicelles of dannacanthal-loaded amphiphilic modified chitosan: Preparation, characterization and cytotoxicity study. *Mater. Sci. Eng., C* **2017**, *77*, 1068−1077.

(27) McDaniel, J. R.; Weitzhandler, I.; Prevost, S.; Vargo, K. B.; Appavou, M.-S.; Hammer, D. A.; Gradzielski, M.; Chilkoti, A. Noncanonical Self-Assembly of Highly Asymmetric Genetically Encoded Polyamide Amphiphiles into Cylindrical Micelles. *Nano Lett.* **2014**, *14*, 6590−6598.

(28) Wang, B. L.; Jin, T. W.; Han, Y. M.; Shen, C. H.; Li, Q.; Lin, Q. K.; Chen, H. Bio-inspired terpolymers containing dopamine, cations and MPC: a versatile platform to construct a recyclable antibacterial and antifouling surface. *J. Mater. Chem. B* **2015**, *3*, S501−S510.

(29) Harada, Y.; Morinaga, Y.; Kaku, N.; Nakamura, S.; Uno, N.; Hasegawa, H.; Izumikawa, K.; Kohno, S.; Yanagihara, K. In vitro and in vivo activities of piperacillin-tazobactam and meropenem at different inoculum sizes of ESBL-producing Klebsiella pneumoniae. *Clin. Microbiol. Infect.* **2014**, *20*, O831−O839.

(30) Signoretto, C.; Marchi, A.; Bertoccelli, A.; Burlacchini, G.; Tessarolo, F.; Caola, I.; Pessati, E.; Zaura, E.; Papetti, A.; Lingstrom, P.; Pratten, J.; Spratt, D. A.; Wilson, M.; Canepari, P. Effects of mushroom and chicory extracts on the physiology and shape of Prevotella intermedius, a periodontopathogenic bacterium. *J. Biomed. Biotechnol.* **2011**, *2011*, 635348.

(31) Anumula, L.; Kumar, S.; Kumar, V. S.; Sekhar, C.; Krishna, M.; Pathapatti, R. M.; Venkata Sarath, P.; Vadagandam, Y.; Manne, R. K.; Mudlapudi, S. An Assessment of Antibacterial Activity of Four Endodontic Sealers on Enterococcus faecalis by a Direct Contact Test: An In Vitro Study. *ISRN Dent.* **2012**, *2012*, 989781.

(32) Rikkou-Kalourkoti, M.; Panteli, P. A.; Patrickios, C. S. Synthesis and characterization of amphiphilic diblock copolymers of 2-(1-imidazolyl)ethyl methacrylate and styrene. *Polym. Chem.* **2014**, *5*, 4339.