

Facile Fabrication of Hyperbranched Polyacetal Quaternary Ammonium with pH-Responsive Curcumin Release for Synergistic Antibacterial Activity

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 Electronic Supplementary Information

Abstract In the current global crisis of antibiotic resistance, pursuit of an effective multi-pathway collaborative approach to enhance antibacterial activity is urgently needed. Here, a kind of hyperbranched polyacetal quaternary ammonium (Hyper-ace-QA) with efficiently antibacterial function were synthesized by a succession of efficient click strategies. The high molecular weights can be obtained just after UV irradiation for 3 h, and the grafting ratio can be easily adjusted through controlling solvent system, molar ratio, and temperature. Cytotoxicity studies indicated that Hyper-ace-QA had good biocompatibility and can be used as a potential antibacterial dressing. More importantly, after *in situ* encapsulation of bioactive curcumin drugs into the hyperbranched intramolecular cavities, the acid-labile acetal linkers within the hyperbranched backbone made the loading antibacterial drugs rapidly release with pH- or bacterial-responsive behaviors since many bacteria can produce acids at the infection site by the combined actions of immune response and bacterial metabolism. Therefore, the integration of quaternary ammonium characteristics and pH-triggered curcumin release could facilitate the antibacterial activity against gram-positive *Staphylococcus aureus*. This work represents a synergistic strategy on offering important guidance to rational design of multifunctional antimicrobial vehicles, which would be promising therapeutic alternatives for first aid treatment of wound infection in critical situations.

Keywords Hyperbranched polymer; Acetal; Quaternary ammonium; pH-Responsive; Antibacterial activity

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INTRODUCTION

Bacterial infection has developed as one of the most significant public health challenges. Although the easy usage of antibiotics can drastically reduce mortality from bacterial infection in clinical medicine, their overuse of antibiotics always causes the generation of antibiotic-resistant bacteria that can kill nearly 700,000 people worldwide each year and severely threaten human's health.^[1–3] Thus, a various therapeutic alternative to antibiotics has been continuously developed for treating bacterial infection and killing the antibiotic resistant bacteria. Unfortunately, most of them display a similar mechanism to traditional antibiotics, resulting in the inevitable emergence of

resistance to novel antibiotics.^[4–8] In the past few decades, many efforts have been focused on exploring the effective infection prevention by mixing with bactericidal additives like polyguanidine, silver, phosphonium or ammonium compounds, etc., but they were associated with leaching, short-term activity, and toxicity.^[9–11] In addition, although natural chitosan is considered as an antibacterial polysaccharide, its moderate bactericidal capacity limited to acidic conditions made itself inefficient.^[12] Therefore, pursuit of multimodal combination therapy is urgently needed for antibiotic-resistant bacteria.

Curcumin is a natural-based polyphenol with excellent biocompatibility and bioactivity from the rhizomes of *Curcuma longa* (turmeric), which has been broadly utilized for the wound treatment due to its effective anti-inflammatory, antibacterial and anti-oxidant activity.^[13–15] However, its poor water solubility and bioavailability significantly hinder the biomedical application. These limitations can be overcome by physical encapsulation of curcumin into polymer-based carriers. Among them, hyperbranched polymer has attracted interest as environmentally friendly materials for their various

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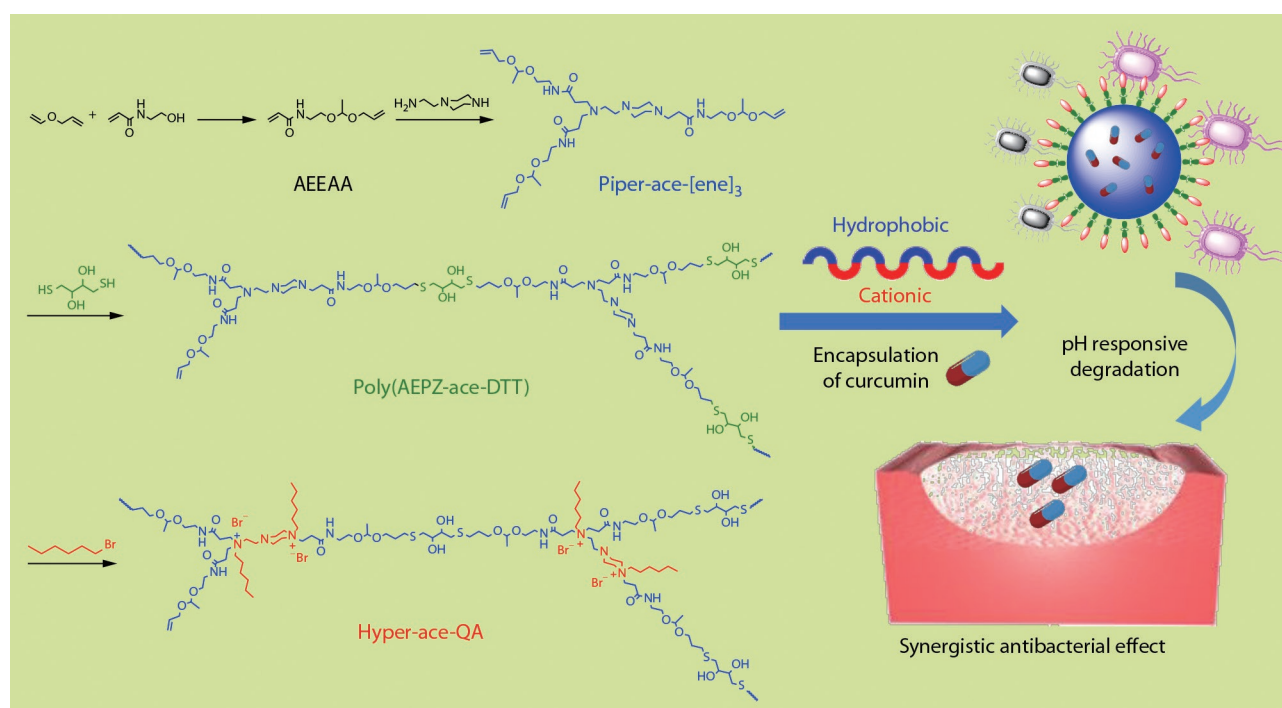
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applications.^[16–18] Although hyperbranched polymers are of irregular shapes, not perfectly symmetrical as dendrimers, they can be easily synthesized *via* a one-pot polymerization. Moreover, their abundant terminal functional groups and numerous interior cavities provided more possibilities on chemical modification and physical encapsulation of hydrophobic drugs to improve the polymeric multifunction and drug solubility, ensuring the continuity of drug release and reproducibility of pharmacokinetic behavior.^[19–22] Wherein, hyperbranched poly(amino-ester)s are a series of biodegradable polymers *via* the Michael addition reaction of primary/secondary amines with acrylate monomers, which possess a large number of tertiary amines groups that can be elaborately designed and alkylated to generate built-in quaternary ammonium salts for antibacterial fields.^[23–27]

Recent developments in drug carriers for antimicrobial therapy have enabled programmable delivery of therapeutics with stimuli-responsive behavior. It is desired for a long time to construct an ideal criterion that the drug delivery system should quickly achieve high drug concentration to kill the bacteria at an early period and exert an environmental response whenever needed to avoid recurrence of infection. Since the low oxygen tension and inflammation at the site of infection can trigger the anaerobic fermentation of bacteria to generate abundant lactic acid and acetic acid, the site of bacterial infection can gradually become the acidic condition with a pH 5.0–5.5.^[28,29] So, pH-responsive drug delivery has therefore showcased the importance on resolving the disadvantages of conventional antibiotics *via* improving the delivery efficiency and maintaining high drug concentration at targeted bacterial infection sites. pH trigger is recognized as an attractive strategy on the targeted antibiotic delivery in the

antimicrobial arsenal, and thereby pH-responsive antibiotics release can suppress the drug resistance and reduce the cytotoxicity.^[30–35] Wherein, acetal linkages being of great interest due to their pH sensitivity and non-acidic metabolites are generally introduced into the polymeric architectures to construct the pH-responsive carriers.^[36,37] Thus, it is highly anticipated that the elaborate fabrication of hyperbranched polyacetal polymers and clear elucidation of pH-responsive curcumin release would be beneficial for the development of smart drug delivery systems with multifarious architectures and desired functions to promote antibacterial efficacy.

In the present work, we prepared a novel class of hyperbranched polyacetal quaternary ammonium polymer (Hyper-ace-QA) through the consecutive aza-Michael addition, thiol-ene click, and Menschutkin click reactions for synergistic antimicrobial effect. The acid-labile acetal linkers at the interior made the loading antibacterial curcumin drugs remain stable under neutral conditions but quickly release from the carriers in acidic environment and/or bacterial compartments (Scheme 1). Under this circumstance, the combination of quaternary ammonium backbone and curcumin transport within the hyperbranched polymers endowed these Hyper-ace-QA@Cur nanoparticles with synergistic antimicrobial effect on that positively charged Hyper-ace-QA could interact with the negatively charged bacterial cell membranes through an electrostatic interaction to kill the bacteria, and meanwhile the generative acidic conditions from bacterial metabolism can cause the cleavage of acetal bonds and enable the curcumin release. This multilevel antibacterial biology could effectively suppress the bacteria growth at an early period and exert an environmental responsive effect whenever needed over a long period, exhibiting intelligent therapeutic



Scheme 1 Synthetic approach of pH-responsive hyperbranched polyacetal polymer (Hyper-ace-QA) and curcumin-loaded nanoparticles (Hyper-ace-QA@Cur) for the synergistic antibacterial therapy.

activity against *S. aureus* and also facilitating the wound healing. Taken together, this current work offers an effective antibacterial therapeutic modality for the treatment of infectious wounds.

EXPERIMENTAL

Materials

Allyl vinyl ether (95%, Alfa Aesar), *N*-(2-hydroxyethyl) acrylamide (98%, TCI), pyridinium *p*-toluenesulfonate (PPTS, 98%, Energy Chemical), dithiothreitol (DTT, 99%, J&K), 1-(2-aminoethyl) piperazine (AEPZ, 98%, Sigma Aldrich), 2,2-dimethoxy-2-phenylacetophenone (DMPA, 99%, J&K), bromohexane (98%, Energy Chemical), *N,N'*-dimethyl formamide (DMF), dimethyl sulfoxide (DMSO), dichloromethane (DCM), methanol (MeOH), ethyl acetate and triethylamine (TEA) (reagent grade, Beijing Chemical Works) and other compounds were purchased from Beijing Chemical Works and used without further purification.

Characterizations

Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker 400 MHz spectrometer. Matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) measurements were carried out using a Bruker BIFLEX III equipped with a 337 nm nitrogen laser. Gel permeation chromatography (GPC) measurements were performed at 50 °C using an SSI pump fitted with a Wyatt DAWN EOS multiangle laser light scattering (MALLS) detector coupled with Wyatt Optilab DSP refractive index detector with 0.02 M LiBr in DMF as the eluent at a flow rate of 1.0 mL·min⁻¹. The molecular weight (MW) and polydispersity (PDI) were calculated from the MALLS signal by Astra software. Transmission electron microscopy (TEM) images were obtained on a JEM-2200FS microscope (JEOL, Japan) after the samples were negative stained by sodium phosphotungstate. Generally, a 3 µL droplet of nanoparticles solution was dropped onto a copper grid (300 mesh) coated with a carbon film, then the excess liquid was wicked off and the grids were immediately placed onto individual droplets of freshly prepared and filtered, 2 wt% aqueous sodium phosphotungstate. After 2 min, excess stain was removed and the grids were allowed to dry thoroughly. Size distribution of the nanoparticles were characterized by dynamic light scattering (DLS) using a zetasizer (Nano-ZS, Malvern, UK) with a 632.8 nm laser light set at a scattering angle of 173°.

Synthesis of *N*-(2-(1-(allyloxy)ethoxy)ethyl) Acrylamide (AEEAA)

N-(2-hydroxyethyl) acrylamide (6.9 g, 60 mmol) and PPTS (0.125 g, 5 mmol) were dissolved in 100 mL of dry DCM at 0 °C under nitrogen by dropwise addition of allyl vinyl ether (4.2 g, 5 mmol) in 20 mL of dry DCM. After stirring at room temperature for 12 h, K₂CO₃ was added to quench the reaction and the mixture was filtered by a pad of celite. Then the filtrate was evaporated under reduced pressure to obtain crude product, which was purified by silica gel chromatography using DCM/ethyl acetate (V/V=15/1) containing 1% (V/V) TEA as eluent to obtain the pale-yellow liquid of acetal monomer AEEAA with 70.2% yield.

Synthesis of Piper-ace-[ene]₃

AEEAA (1 g, 5.02 mmol), AEPZ (0.172 g, 1.34 mmol) and TEA (242 mg, 2.4 mmol) were dissolved in 5 mL of MeOH/H₂O (V/V=7/3). After stirring at 70 °C for 48 h, the mixture was diluted by DCM

followed by washing with brine for three times. The organic layer was dried with MgSO₄ and concentrated, which was then purified by silica gel chromatography using ethyl acetate containing 1% (V/V) TEA as eluent to obtain the pale-yellow liquid of Piper-ace-[ene]₃ with 80.1% yield.

Synthesis of Poly(AEPZ-ace-DTT)

Piper-ace-[ene]₃ (0.2 g, 0.275 mmol), DTT (64 mg, 0.413 mmol) and DMPA (32 mg, 0.124 mmol) were dissolved in 5 mL of MeOH. The mixture was purged with argon for 10 min and irradiated under a 365 nm UV lamp at room temperature for 3 h. The crude product was precipitated in diethyl ether for three times to obtain the yellow liquid of poly(AEPZ-ace-DTT) with 70.2% yield.

Synthesis of Hyperbranched Polyacetal Quaternary Ammonium Hyper-ace-QA

Poly(AEPZ-ace-DTT) (66 mg, 0.14 mmol) and bromohexane (0.23 g, 1.4 mmol) were dissolved in 5 mL of DMF at 55 °C for 3 days. The crude product was precipitated in hexane/diethyl ether (V/V=1/1) for three times to obtain the origin-yellow liquid of Hyper-ace-QA with 80.3% yield.

pH-Responsive Degradation of Hyperbranched Polyacetal Polymers

The pH-responsive degradation of the polyacetal hyperbranched polymers was investigated *via* dissolving in deuterated phosphate buffer at pH=5.0. The ¹H-NMR spectra of hyperbranched polymers were recorded at different time intervals and degradation profiles were calculated from the integration of the peaks at 1.31 ppm due to the methyl in the acetal groups.

Self-assembly of Hyper-ace-QA Micelles

Polymeric micelles were prepared by a dialysis method. A typical self-assembly aggregate was prepared as following: Hyper-ace-QA polymer (5 mg) was dissolved in methanol (1 mL), and then deionized water (4 mL) was added dropwise into the solution at the rate of 0.05 mL/min *via* a syringe pump. The colloidal dispersion was further stirred for another 2 h with the fixed temperature at 30 °C during the self-assembling process. The organic solvent was removed by dialysis (MW cutoff, 3.5 kDa) against deionized water for 3 days, and collected by the lyophilisation method, which was stored at 4 °C for the following experiments.

Formation of the Curcumin-loaded Nanoparticles

The Hyper-ace-QA@Cur nanoparticles were prepared as follows: Hyper-ace-QA and a certain amount of curcumin drugs were dissolved in 1 mL of DMF. Then, 4 mL of deionized water was added dropwise into the solution at the rate of 0.05 mL/min *via* a syringe pump. Subsequently, the solution was dialyzed against deionized water to remove the unassembled macromolecules and other by-products. After dialysis for 3 days with a dialysis membrane tube (MW cutoff, 3.5 kDa), the remaining solutions were frozen in liquid nitrogen and collected by the lyophilisation method, which was stored at 4 °C for the following experiments.

pH-Responsive Degradation of the Nanoparticle

For the self-assembled Hyper-ace-QA and Hyper-ace-QA@Cur nanoparticles, pH-responsive degradations were investigated by adding 100 µL of solution to 1.5 mL of phosphate buffer with

a pH of 5.0 and then recording the morphological variation by TEM and the size distribution by DLS.

In vitro Drug Release Behavior of Hyper-ace-QA@Cur Nanoparticles

Curcumin release from Hyper-ace-QA@Cur nanoparticles was investigated as follows: 4 mg of curcumin-loaded nanoparticles were dispersed into 2 mL of phosphate buffer with a pH of 7.4 and an acetate buffer with a pH of 5.0, respectively. Then the suspensions were loaded into dialysis tube (MW cutoff, 3.5 kDa) with continuous shaking at 37 °C. After adding 20 mL of release medium containing 0.5 wt% of the Span 80, 3 mL of sample solution was withdrawn and replaced by an equal volume of fresh medium to keep constant volume. For pre-desired time interval, the released curcumin was monitored on a UV-visible spectrophotometer. The cumulative release ratio (R_i) of curcumin concentration was calculated by the following equations:

$$\begin{cases} R_i = \frac{V_0 C_i}{m_{\text{drug}}} \times 100\% \quad (i = 1) \\ R_i = \frac{V_0 C_i + V_e \sum_{j=1}^{i-1} C_{j-1}}{m_{\text{drug}}} \quad (i \geq 2) \end{cases} \quad (1)$$

where the R_i represents the accumulative release percent of loading curcumin, and the m_{drug} is the total amount of curcumin within the Hyper-ace-QA@Cur nanoparticles, V_0 and V_e mean the volume of the total release medium and the renewed medium, respectively. C_i was the curcumin drug concentration in the release medium that taking out at the i^{th} time. The release experiments were preceded in triplicate.

Antibacterial Experiments

To investigate the antibacterial ability, Hyper-ace-QA@Cur nanoparticles were incubated with *S. aureus* with various incubation concentration. Specifically, Hyper-ace-QA@Cur nanoparticles were firstly placed in a sterilized distilled water at various concentrations of 500 mg/mL, 250 µg/mL and 125 µg/mL in 45–55 °C. Then, various concentrations of Hyper-ace-QA@Cur were added into the plate containing the nutrient AGAR medium with continuous shaking to mix the Hyper-ace-QA@Cur solution well with the medium. Then, 2 µL of *S. aureus* suspension (1×10^7 CFU/mL) was dropped and seeded on the medium plate containing antibacterial solution, and the bacterial circle was formed with the diameter of 5–8 mm. Nutrient AGAR plates without Hyper-ace-QA@Cur nanoparticles were inoculated in the same way and used as positive controls. Finally, the inoculated plates were placed in a constant temperature incubator at 35 °C and further incubated for 24 h to observe the growth of colonies and evaluate the antibacterial ability. Each group was performed in triplicate.

In vitro Cytotoxicity

In vitro cytotoxicity assays of Hyper-ace-QA was evaluated by CCK-8 assay. The model cells were seeded into 96-well culture plates at a density of 1×10^4 cells/100 µL per well, and incubated in 200 µL of Hyclone Dulbecco's Modified Eagle Medium (DMEM) with 10% fetal bovine serum (FBS) in a 5% CO₂ incubator at 37 °C for 24 h. The culture medium was replaced by 200 µL of complete DMEM medium containing 20 µL of various concentrations of Hyper-ace-QA. After culturing for 48 h, the medium was substituted by 100 µL of new medium and 10 µL of CCK-8. Subsequently, the cells were incubated at 37 °C for

another 3 h. Then the cell viability was evaluated by comparing the absorbance of measured solutions by a micro-plate reader at 450 nm. Cells cultured in DMEM medium containing 10% FBS (without exposure to sample) were used as controls.

RESULTS AND DISCUSSION

Synthesis and Characterization of Hyperbranched Polyacetal Polymers

The specific synthetic route is shown in Scheme 1. Since the AEEAA monomer containing an acid-labile acetal group was facilely synthesized by the click-type reaction of hydroxyl and vinyl ether in the presence of PPTS (Fig. S1 in the electronic supplementary information, ESI), the Piper-ace-(ene)₃ could be easily prepared with AEPZ using the efficient azo-Michael addition reaction. ¹H-NMR spectrum showed the explicit structural attributes with the typical acetal groups at 4.71 and 1.27 ppm and precisely integrated area ratio compared to other peaks, certifying the successful synthesis of alkene-terminated Piper-ace-(ene)₃ monomer (Fig. 1A). In addition, MALDI-TOF MS was also utilized to verify the architecture in Fig. 1(B), which exhibited the target peaks in agreement with its theoretical M_w s, further demonstrating the well-defined structure and high purity by the feasible azo-Michael reaction. Once upon treatment with the thiol-terminated DTT monomer, the peripheral alkenes of Piper-ace-(ene)₃ could efficiently and selectively reacted with the thiol to obtain the hyperbranched polymer of poly(AEPZ-ace-DTT) via a thiol-ene “click” polymerization using the ‘A₂+B₃’ strategy. Fig. 1(C) clearly indicated the assignment of the hyperbranched topology with the gradual disappearance of vinyl groups and the generation of typical resonance peaks of DTT. Subsequently, on account of abundant tertiary amines within the poly(AEPZ-ace-DTT), high-efficient quaternization of bromohexane could readily yield the hyperbranched polyacetal quaternary ammonium of Hyper-ace-QA without any tedious purification steps. The occurrence of characteristic peak at 0.8 ppm in the ¹H-NMR (Fig. 1D) indicated that bromohexane was successfully reacted with the tertiary amine and the persistently stable acetal peak at 4.72 ppm proved the progress of Menschutkin reaction. This Menschutkin click chemistry not only retained the pH-responsive degradation but also endowed the hyperbranched polymers with excellent water-solubility and favourable antibacterial activity.

To obtain soluble polymers in the A₂ + B₃ approach, the polymerization needs to be terminated before the gel point, which requires adjustment of the reaction conditions (catalyst content, temperature, reaction time, etc.) and adjustment of the monomer concentration and feed ratio of A/B group. We firstly adopt 12 wt% of catalyst to improve the efficiency of thiol-ene polymerization to avoid the crosslinking of residual vinyl groups during the UV irradiation. In addition, the reaction temperature was controlled under 30 °C and the reaction time was set at 3 h to avoid the occurrence of side reaction. Moreover, taking consideration into the probably oxidative coupling thiol groups to generate the disulphide bonds during the polymerization, an unequal molar ratio of ene/thiol groups may impair the polymerization process and decline the molecular weight of hyperbranched polymers. Therefore, we tried to adjust the ene/thiol molar ratios (1/1, 1/1.5 and 1/2) to improve its polymerization degree with fixed illumina-

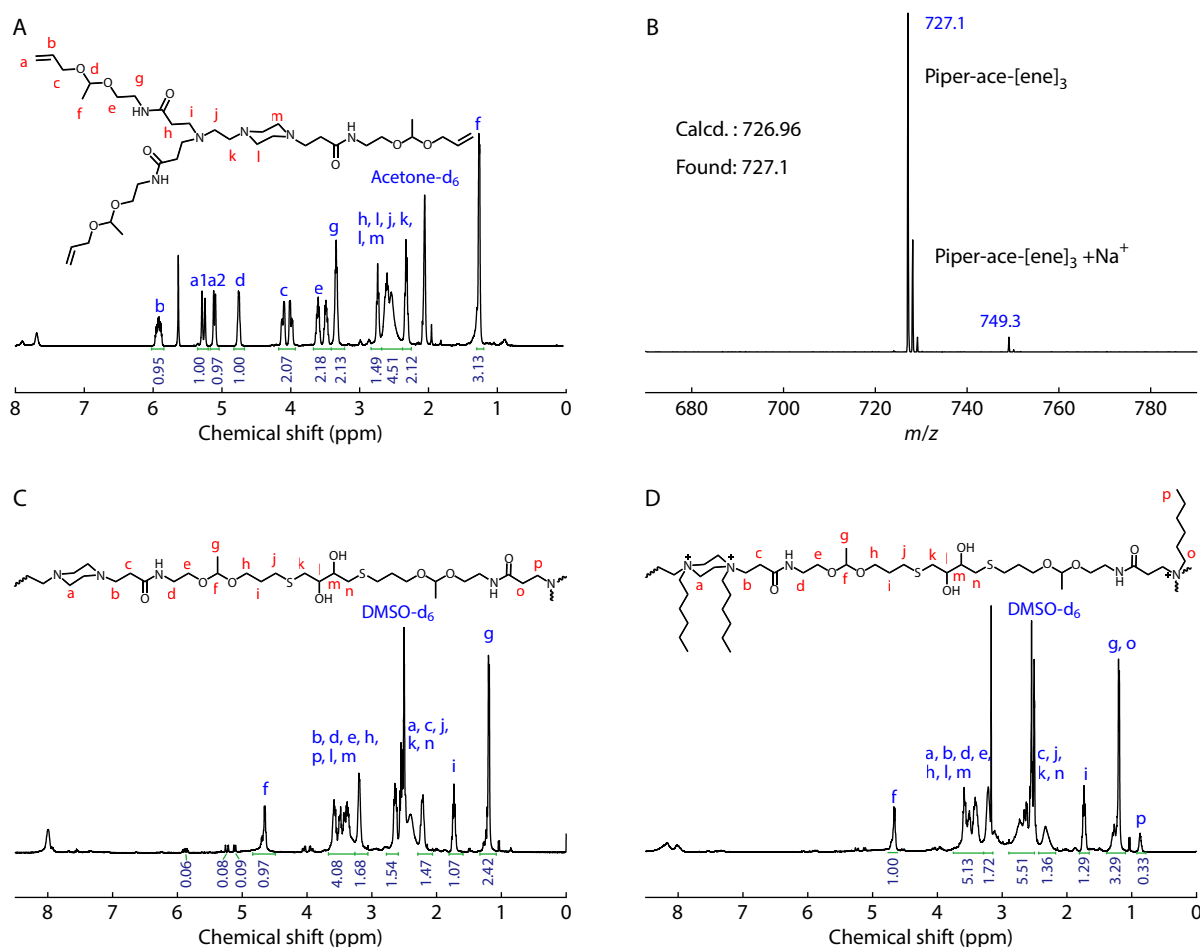


Fig. 1 (A) ^1H -NMR and (B) MALDI-TOF MS spectra of Piper-ace-(ene) $_3$ monomer; (C) ^1H -NMR spectrum of hyperbranched poly(AEPZ-ace-DTT) polymer; (D) ^1H -NMR spectrum of hyperbranched polyacetal quaternary ammonium of Hyper-ace-QA polymer.

tion time of 3 h, because the molecular weight was direct evidence to disclose the nature of hyperbranched frameworks that was closely relative to the polymeric properties. On account of the messy and confused alkene variation in the polymerization process, we used the integrated area ratio of typical acetal peak (blue b) and emerged characteristic methylene (blue a) to quantify the degree of thiol-ene reaction, which could indicate the remaining proportion of vinyl groups within the hyperbranched polymers. As shown in Fig. 2(A), increasing the thiol concentration could indeed consume more vinyl groups but the molecular weight became lower from 82100 to 19400 and PDI decreased from 2.56 to 1.24 (Fig. 2B and Table S1 in ESI), which was due to that the excessive thiol could cause the premature termination of double bond. This result indicated the efficient thiol-ene reaction with the negligible oxidation loss of thiol groups during the UV polymerization.

We further used bromohexane to modify the structure of tertiary amines and found that the solvent system, reactive time, and temperature played important roles in the reaction efficiency. Compared to the low polarity of solvent system (e.g., acetone) to reduce the reaction efficiency, we applied the stronger polar DMSO solvent for the Menshutkin reaction. However, the acetal structures within the hyper-

branched polymer were unexpectedly broken, which may ascribe to that the remaining bromohexane further reacted with secondary amine in the system and thus generating hydrogen bromide (HBr) to promote the acetal degradation. Therefore, the DMF solvent was chosen as the most appropriate moderate polarity without the acetal fracture and skeleton destruction in the same conditions. To clearly quantitative analysis of the relationship of reaction time and Menshutkin efficiency, Piper-ace-(ene) $_3$ monomer was employed to react with the bromohexane for precise calculation of integrated areas because of the complex and inconvenient attribution peaks of poly(AEPZ-ace-DTT) polymer. As shown in Fig. 2(C), with the prolongation of the reaction time, the Menshutkin reaction efficiency almost did not change with the characteristic peak at 0.8 ppm after 3 days, indicative of the setting reaction time of 3 day. In addition, we also explore the effect of temperature on structural stability of hyperbranched polymer and found that high reactive temperature at 59 °C could produce the unpredictable side reactions to impair structural integrity. Fig. 2(D) showed that some characteristic peak changes at 3.0–4.0 ppm had been changed compared to that of 55 °C, suggesting that this hyperbranched polymer was degraded to a certain degree and confirming the set temperature of 55 °C.

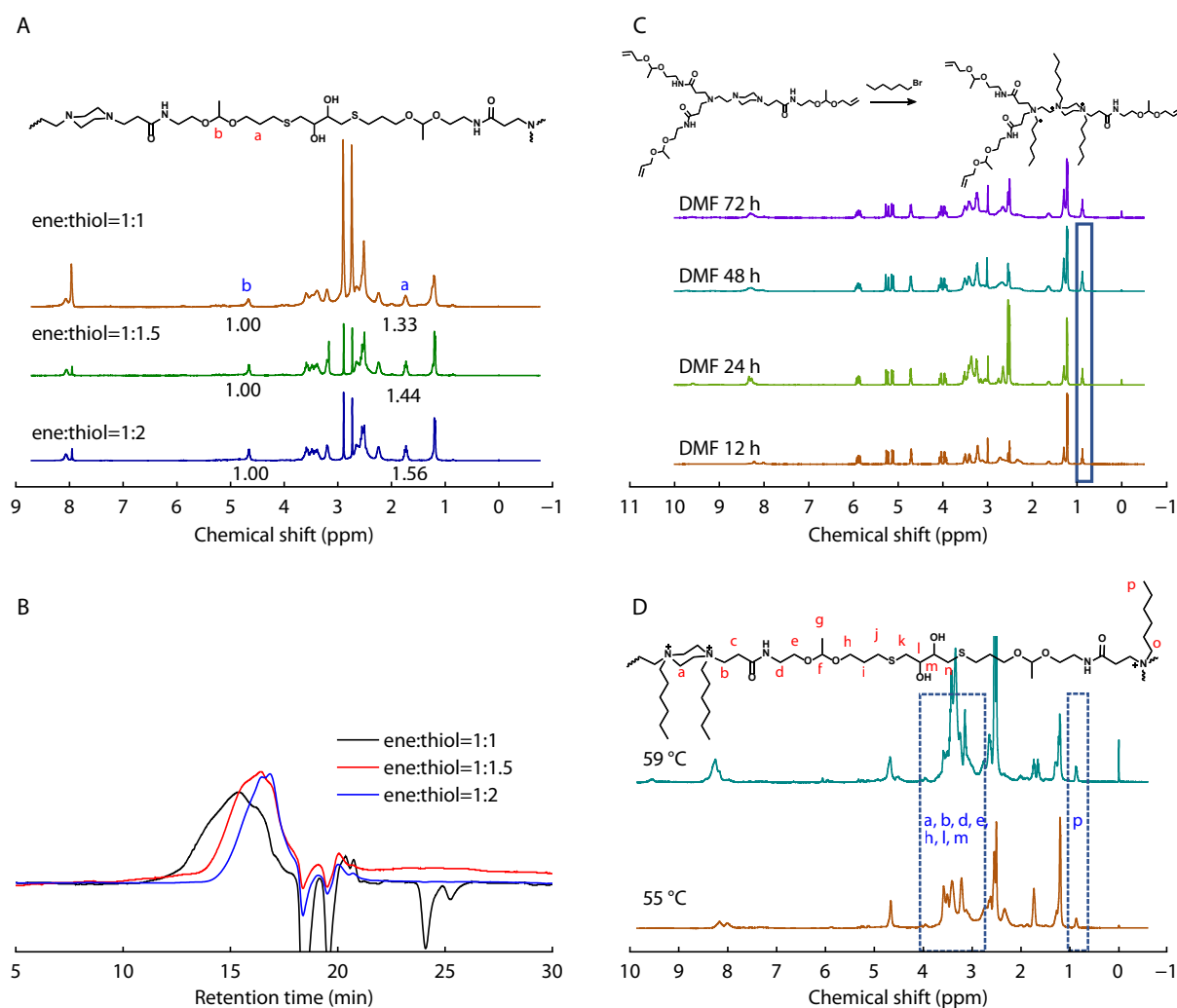


Fig. 2 (A) ^1H -NMR spectra and (B) GPC trace of poly(AEPZ-ace-DDT) polymer with various ene/thiol molar ratios; (C) ^1H -NMR spectra of Piper-ace-QA with various reactive time; (D) ^1H -NMR spectra of hyperbranched Hyper-ace-QA polymer with various temperatures.

Degradation Properties of the Hyper-ace-QA Polymer

The presence of acetal groups within the hyperbranched polymers makes them easy to hydrolyse under faintly acidic conditions. The degradation behavior of the soluble Hyper-ace-QA was investigated in pH 5.0 phosphate buffer (D_2O), and the ^1H -NMR spectra were recorded at different time intervals. As shown in Fig. 3(A), although the peaks attributed to methyne in the acetal groups were concealed by solution peaks, gradual disappearance of the peaks at 1.31 ppm corresponding to the methyl in the acetal groups and the appearance of new peaks at 9.8 ppm and 5.2 ppm attributed to the degraded acetaldehyde were observed. The degradation percentage was calculated from the integration of the peaks corresponding to the acetal groups and degradation profiles of the Hyper-ace-QA polymer are summarized in Fig. 3(B). It was mentioned that hyperbranched Hyper-ace-QA polymer hydrolysed at a relatively slow rate of 65% degradation at 500 h, probably because its compact structure could prevent the protons permeation to a great extent. Then, we illustrated this pH-triggered degradation behavior. In fact, the appearance of peak *a* proved that existence of hemiacetal degradation while the appearance of peak *b* proved the acetal formation for complete

degradation. Both these two degradation modes simultaneously existed during the whole degradation process (Fig. 3A), which may lead to the diversification of Hyper-ace-QA degradation in solution and form the irregular aggregate products in a more complex way, thus slowing down the degradation rate even in the acidic pH conditions.

Preparation and pH-Responsive Degradation of the Hyper-ace-QA@Cur Nanoparticles

Hyper-ace-QA polymer could self-assemble into micellar nanoparticles in aqueous solutions because of its hydrophilic quaternary ammonium and hydrophobic alkyl chain, which was investigated by TEM and DLS in Figs. 4(A-a) and 4(B-a). These nanoparticles exhibited the spherical morphology with the dimension of 35 nm, but DLS result showed a particle size of 165 nm with a polydispersity index (PDI) of 0.32, which indicated its interior space for potential encapsulation of small molecules. Curcumin, as a hydrophobic polyphenolic compound extracted from the rhizomes of turmeric plants, has been categorized as 'Generally Regarded as Safe' (GRAS) by the U.S. Food and Drug Administration, which had demonstrated several pharmacological and biological

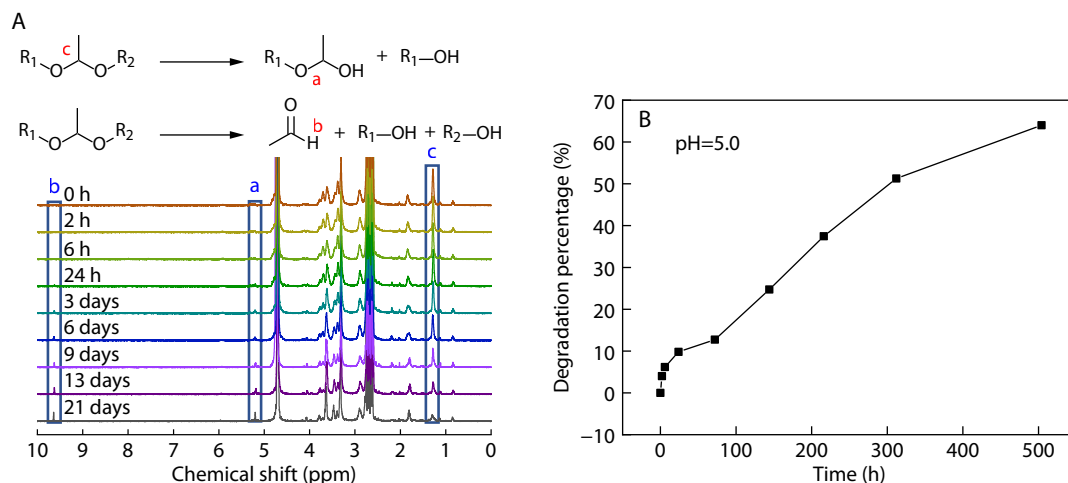


Fig. 3 (A) ^1H -NMR spectra of Hyper-ace-QA polymer at pH 5.0 for various times. (B) Degradation kinetics plots of the Hyper-ace-QA polymer at pH 5.0.

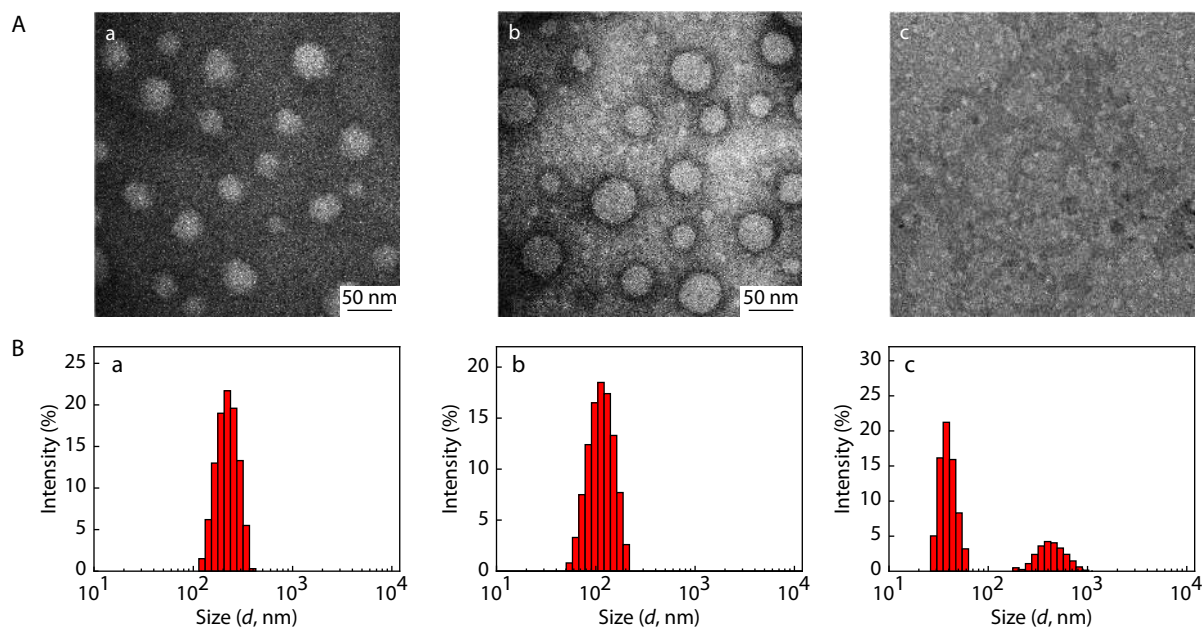


Fig. 4 (A) TEM images and (B) size profiles of the Hyper-ace-QA (a) and Hyper-ace-QA@Cur nanoparticles before (b) and after (c) treatment of pH 5.0 solutions for 8 h.

activities such as anticancer, antibacterial and antioxidant properties, but its biomedical application is limited by its poor water solubility and bioavailability. Therefore, free curcumin was further *in situ* encapsulated into the polymeric micelles to form the Hyper-ace-QA@Cur nanoparticles, which exhibited the larger average hydrodynamic size of 46 nm using TEM and 244 nm by DLS in Figs. 4(A-b) and 4(B-b). These aggregated nanoparticles possessed good storage stability that was important for the drug formulations although there was some degree of micellar swelling in aqueous solutions, however, they were easy to disassemble under faintly acidic condition because of the acid-labile acetal linker within the aggregates. After incubation in pH 5.0 PBS solutions for 8 h, Fig. 4(A-c) shows the morphological collapse and degradation in a certain degree, and the small molecules and aggregates are observed in Fig. 4(B-c), confirming pH-induced cleavage of acetal bonds and

dissociation of the aggregates. Therefore, these pH-sensitive nanoparticles could keep stable at normal physiological environments and degradable in response to acidic conditions, which may be used as the intelligent carriers in biomedical fields.

***In vitro* Biocompatibility, Drug Release Behavior and *In vitro* Antimicrobial Assay**

CCK-8 assay was used to evaluate the *in vitro* cytotoxicity of Hyper-ace-QA nanoparticle. After incubation for 24 h using NIH 3T3 cells, cell viability was more than 95% until the tested concentration reached 300 $\mu\text{g}/\text{mL}$, demonstrating its good biocompatibility and usability (Fig. 5A). The drug loading rate of the Hyper-ace-QA@Cur nanoparticles was 9.4%, which may ascribe to the difficulty on the encapsulation of extremely hydrophobic curcumin drug into the hydrophilic Hyper-ace-QA nanoparticles. Since the acid-labile acetal linker was easy to

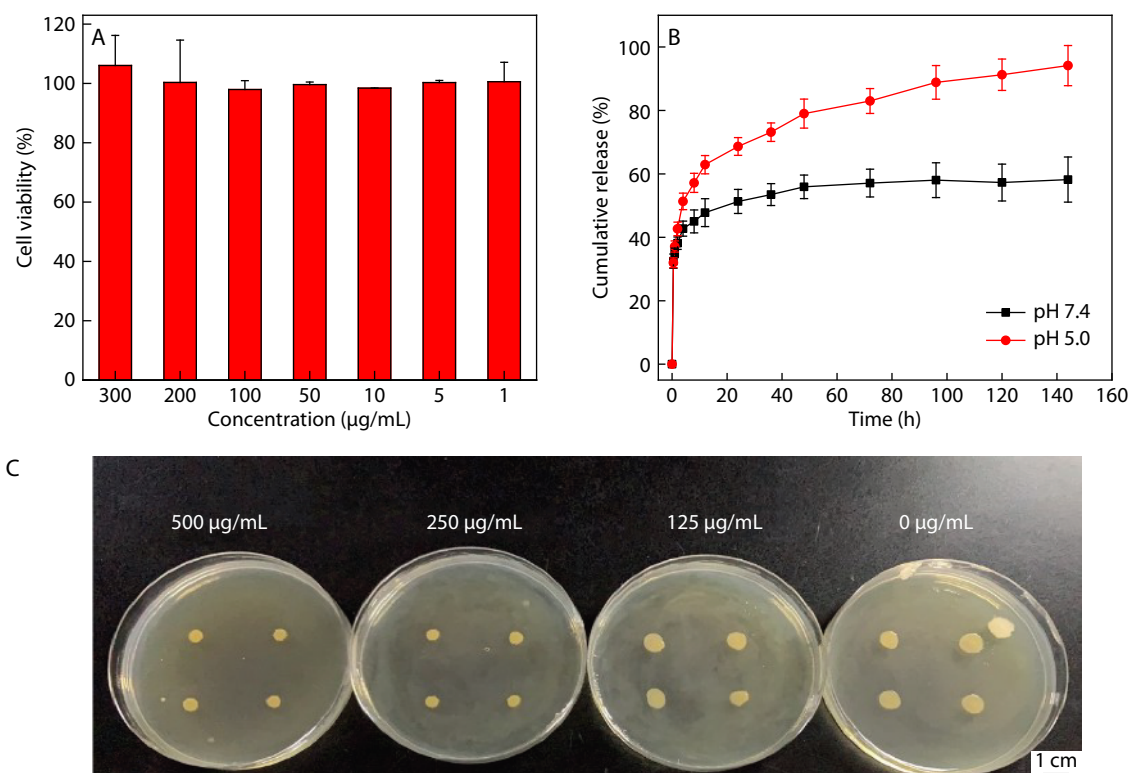


Fig. 5 (A) Cytotoxicity of NIH 3T3 cells following 24 h incubation with Hyper-ace-QA nanoparticles; (B) pH-triggered drug release from the Hyper-ace-QA@Cur nanoparticles after treatment in PBS (pH 7.4 and 5.0) solutions; (C) Representative inhibition zone of *S. aureus* treated with Hyper-ace-QA@Cur nanoparticles of different concentrations.

disassemble under faintly acidic condition, we assessed the drug release profiles of Hyper-ace-QA@Cur nanoparticles at the physiological (PBS, pH 7.4) and acidic conditions (PBS, pH 5.0) to simulate the severe infection environment. As shown in Fig. 5(B), there was a significant initial bursting release of curcumin even at pH 7.4, which was attributed to the addition of surfactants with a span 80 to increase the solubility of curcumin for accurate measurement in aqueous solutions. In this case, several hydrophobic drugs were lightly attached to the surface of nanoparticles. While treatment in harsh pH 5.0, accumulative curcumin release was dramatically accelerated to reach the peak at day 6, which was attributed to the cleavage of acetal linker to accelerate the drug release upon exposure into acidic environment. Moreover, the solubility of curcumin in acidic pH can be significantly improved to accelerate the drug release. Meanwhile, the polymeric protonation, internal structural change and electrostatic repulsion effects may also influence this drug release behavior compared to the physiological environments. Besides, the initial burst release of 40% content and following continuous curcumin release for more than 6 days suggested their enhanced antibacterial activity in clinical application scenarios that required the high concentration of antibiotics on the effective inhibition of bacteria growth from infected wounds or locally continuous drug release for a long-term prevention of wound reinfection in the neutral environment. Therefore, in contrast to the conventional curcumin formulations with poor stability and insufficient therapeutic dose, these drug-loaded nanoparticles integrating the quaternary ammonium groups and pH-responsive drug

release could achieve the specific target transport and spatiotemporally controlled release to reduce the drug-resistance and significantly promote the synergistic antibacterial therapeutic effect.

To verify the antibacterial property against *staphylococcus aureus* (*S. aureus*), different concentrations (500, 250, 125 and 0 μg/mL) of Hyper-ace-QA@Cur nanoparticles were mixed and dissolved in medium by AGAR gradient dilution method in this experiment, and then the bacteria were seeded for observing the growth of bacteria to evaluate the antibacterial properties. The results showed an effective antibacterial activity of Hyper-ace-QA@Cur nanoparticles at the concentrations of 250 μg/mL, approximately equal to the curcumin concentration of 23.5 μg/mL. The antibacterial mechanism of this hyperbranched polyacetal quaternary ammonium was due to that positively charged Hyper-ace-QA could interact with the negatively charged bacterial cell membranes through an electrostatic interaction to kill the bacteria. In addition, the generative weak acidic conditions from bacterial metabolism further led to the cleavage of acetal bonds and enabled the curcumin release from the disassembled nanoparticles, which could decrease the chance of infection and promote wound healing.

CONCLUSIONS

In summary, a series of hyperbranched quaternary ammonium polymer with good biocompatibility and synergistic antibacterial ability *via* successively efficient Michael addition,

thiol-ene and Menschutkin reactions. The presence of a lot of acetal bonds made the intelligent degradation under acidic conditions, which in turn enabled the responsive release of encapsulated antimicrobial drugs in an acidic condition, laying a solid foundation for its application prospects in bacterial infection environments. With the aid of the negatively charged bacterial cell membranes through an electrostatic interaction, the curcumin-loaded nanoparticles could perform the multilevel antibacterial biology that suppressed the bacteria growth at an early period and released the antibacterial drugs for a long period. This work demonstrated a hyperbranched polyacetal quaternary ammonium to combat bacteria with the combination of bacteriostatic and bactericidal effect, which will open a new path to design promising potential therapeutic alternatives for bacterial infection.

NOTES

The authors declare no competing financial interest.



Electronic Supplementary Information

Electronic supplementary information (ESI) is available free of charge in the online version of this article at <http://doi.org/10.1007/s10118-022-2884-0>.

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