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Synthesis, characterization, and antibacterial activity of novel amine *N*-halamine-grafted silica nanoparticles with core-shell structure

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ABSTRACT

Novel amine *N*-halamine-grafted silica nanoparticles with core-shell structure were facilely fabricated by grafting polymeric amine *N*-halamine on silica nanoparticles for fighting against pathogenic bacteria. The products were characterized by different techniques such as transmission electron microscopy (TEM), scanning electron microscopy (SEM), and the modified iodometric/thiosulfate technique. The antibacterial activity of the products was verified by selecting Gram-positive bacteria *S. aureus* and Gram-negative bacteria *E. coli* as models via the plate counting method and zone of inhibition study. The antimicrobial results showed that the products possess powerful bactericidal capability against both model pathogen bacteria. Such an investigation opens up a novel idea of making them the potent candidates for deactivating bacteria or even disease control.

KEYWORDS

Polymeric *N*-halamine; Silica; Core-Shell structure; Antibacterial.

INTRODUCTION

Bacterial contamination problem has become a widespread concern worldwide[1-3]. In response to these serious microbial threats, various kinds of antibacterial materials such as metal oxide, quaternary ammonium/phosphonium salts, peptide, guanidine, and *N*-halamine have been developed recently for human survival and environmental protection. Among them, *N*-halamine with one or more nitrogen-halogen covalent bond in structure is one of the most significant antibacterial materials due to their inherent advantages such as powerful antibacterial activity, long-term stability, high durability, and regenerability[4-6].

N-Halamine is always synthesized by halogenating their corresponding precursor amine/amide/imide compounds[7]. Past few decades witnessed increasing amide and imide *N*-halamine, yet there is quite limited report regarding amine *N*-halamine. Herein, 2,2,6,6-tetramethyl-4-piperidinol (TMP) with a structurally hindered amine in structure is utilized as *N*-halamine precursor to prepare amine *N*-halamine[8]. After chlorination treatment, TMP can be readily transformed into amine *N*-halamine for biocidal applications.

It is widely acknowledged that antibacterial activity of *N*-halamine is dependent on the activated surface area. *N*-Halamine with nanometer-size possesses overwhelming superiority than their bulk counterparts[9]. Thus synthesizing nanosized *N*-halamine is an advisable approach for enhancing antibacterial activity. Colloidal template technique is an effective, simple, and general method for obtaining morphology and size governable nanostructures[10]. Silica nanoparticles is the most ideal candidate template because they are nearly nontoxic, biocompatible, chemically inert, optical transparent, optimistically water dispersible and surface functions as the silanol groups on the silica surface offer versatile possibilities for covalently functionalizing the silica-coated particles[11].

In this study, we synthesized novel nanosized amine *N*-halamine nanoparticles with core-shell structure by coating silica nanoparticles with amine *N*-halamine polymer. The photo and chemical structure of the *N*-halamine-grafted silica nanoparticles are shown in Figure 1. The amine *N*-halamine polymer was obtained from their corresponding precursor allyl functionalized TMP, 4-(allyloxy)-2,2,6,6-tetramethylpiperidine (ATMP). Because of the autoinhibition effect of allyl group during the radical polymerization, styrene (St) was introduced to copolymerize with ATMP to produce amine *N*-halamine copolymer coating on silica surfaces[12]. The products were characterized by transmission electron microscopy (TEM), scanning electron microscopy (SEM), and the modified iodometric/thiosulfate titration technique. Antibacterial tests illustrated that the products have excellent bactericidal activity against both Gram-positive and Gram-negative bacteria.

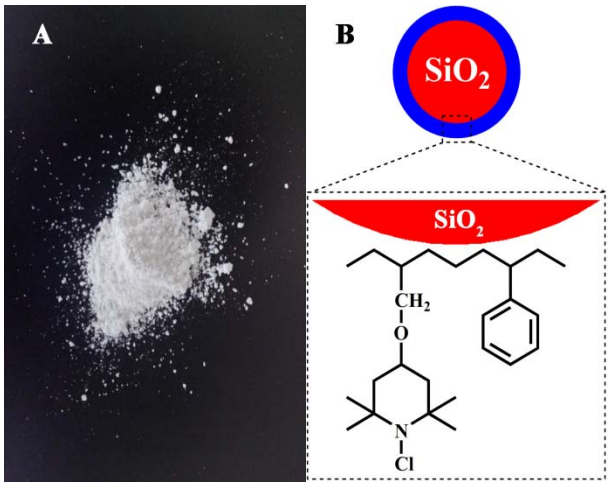

Figure 1 : The photo (A) and chemical structure (B) of the *N*-halamine-grafted silica nanoparticles.

EXPERIMENTAL SECTION

Materials

2,2,6,6-Tetramethyl-4-piperidinol (TMP) was purchased from Nangong Shenghua Chemicals Co., Ltd. Tetraethoxysilane was obtained from Tianjin Guangfu Fine Chemical Research Institute. 3-(Methacryloyloxy)propyl trimethoxysilane and potassium persulfate were available from Shanghai Chemical Reagent Plant. Ammonia and ethanol were purchased from Beijing Chemical Company. Styrene was available from Tianjin Chemical Reagent Plant. Sodium hypochlorite was provided from Sinopharm Chemical Reagent Co., Ltd.

Characterization

The morphology, particle size, surface state, and particle size distribution of the products were captured on a Shimadzu SSX-550 field emission scanning electron microscope (SEM) at 15.0 kV and a Hitachi H8100 transmission electron microscope (TEM) at 200 kV. The samples were dispersed in ethanol with assistance of sonication and casted onto the silicon wafer for SEM and copper grid for TEM characterization, and then dried at room temperature before examination.

Preparation of *N*-halamine-grafted silica nanoparticles

ATMP was synthesized according to the previous report[8]. Tetraethoxysilane was added into a mixture of 40 mL of ethanol, 50 mL of deioned water and 30 mL of ammonia. The mixture was stirred vigorously at room temperature for 24 h to obtain colloidal SiO_2 particles. Then, 2 mL of 3-(methacryloyloxy)propyl trimethoxysilane was added dropwise into the above solution over a reaction period of 24 h. The as-prepared MPS-SiO_2 was dispersed by sonication in 100 mL of distilled water, and 0.01 g of potassium persulfate was introduced into the above mixture. The ATMP/St mixture was added into the above solution, and the radical copolymerization of ATMP with St proceeded on the surface of the silica templates at 75 ^{o}C for 24 h. The as-synthesized poly (ATMP-*co*-St)-grafted silica nanoparticles were immersed in the sodium hypochlorite solution at room temperature for 12 h. The as-prepared *N*-halamine-grafted silica nanoparticles were washed thoroughly with distilled water and dried at 40 ^{o}C to remove any remaining free chlorine from the surface of the sample.

Antimicrobial evaluation

Staphylococcus aureus (*S. aureus*, ATCC 25923, Gram-positive) and *Escherichia coli* (*E. coli*, ATCC 25922, Gram-negative) were used as model bacteria to test the antibacterial activities of the products. Bacteria were grown overnight at 37 ^{o}C under agitation in Luria Bertani growth medium. Cells were harvested by centrifugation, washed twice with phosphate-buffered saline, and diluted to concentrations of 10^{6-7} CFU/mL. The products were dispersed in 0.45 mL sterilized distilled water, vortexed, and then sonicated for 30 min. For antibacterial test, 50 μL of bacteria suspension was added into 450 μL sample suspension, mixed well, and incubated under constant shaking. After a certain period of contact time, 4.5 mL of 0.03 wt % sodium thiosulfate aqueous solution was added into the reaction suspension to stop the antibacterial action of sample. The resulting mixture was mixed well, serially diluted, and then 100 μL of each dilution was dispersed onto LB agar plates.

The inhibition zone study was also performed by a modified Kirby-Bauer technique[13]. The surface of Luria Bertani agar plate and tryptic soy agar plate was overlaid with 1 mL of 10^{8-9} CFU/mL of *S. aureus* and *E. coli*. The plates were then allowed to stand at 37 ^{o}C for 5 min. The products were placed tightly onto the surface of the bacteria-containing agar plate. After incubation at 37 ^{o}C for 24 h, the inhibition zone around the sample was determined.

RESULTS AND DISCUSSION

Characterization of *N*-halamine-grafted silica nanoparticles

The *N*-halamine-grafted silica nanoparticles were synthesized by grafting amine *N*-halamine on the surface of the silica nanoparticles. Pure silica nanoparticles were fabricated by the typical sol-gel approach based on the hydrolysis of TEOS, relying on the well-known stöber process[14]. MPS acts as a silane coupling agent to modify the surface of the silica nanoparticles to obtain terminal C=C bonds on the surface of silica nanoparticles, which is essential to realize the encapsulation of silica nanoparticles with amine polymers via the radical polymerization. A copolymer coating on the surface of silica nanoparticles appeared to give the silica-polymer core-shell nanoparticles. After treatment with sodium hypochlorite, the amine groups in polymer shell were transformed into amine *N*-halamine.

SEM and TEM were applied to characterize the morphology, structure, shape, surface state, size, and particle size distribution of the *N*-halamine-grafted silica nanoparticles. Figure 2A illustrated the SEM image of the *N*-halamine-grafted silica nanoparticles are qusi-monodisperse, spherical-like, and relative smooth spheres. Such a smooth appearance is similar with that of the polystyrene-grafted silica nanoparticles reported previously[15]. From which, we can conclude that the introduction of ATMP component has no significant effect on the polymer coating on the silica surface. The polymer coating is clarified by TEM images in Figure 2B. The *N*-halamine-grafted silica nanoparticles have legible spherical shape and clear core-shell structure. The strong contrast difference between a dark inner center and relatively light edge successfully verifies the coating of silica nanoparticles with amine *N*-halamines. The silica nanoparticles have an average diameter of 475.5 nm and an average polymer coating of 29.4 nm.

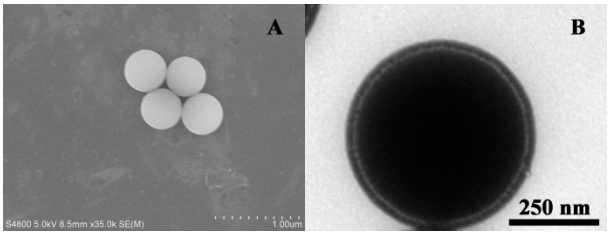

Figure 2 : SEM and TEM image of the *N*-halamine-grafted silica nanoparticles.

Besides morphological characterization, the functional N-Cl group of the *N*-halamine-grafted silica nanoparticles was also proven via the modified iodometric/thiosulfate method. The oxidation-reduction reactions involved in the iodometric/thiosulfate titration are summarized in Figure 3. Positive chlorine in the *N*-halamine-grafted silica nanoparticles suspension oxidized iodide ions to produce iodine to show yellow suspension. The iodine then reacted with starch solution via the chromogenic reaction to give a blue solution. The iodine was exhausted finally by the added thiosulfate to return back to original quasi-transparent solution. Such a color change well verified the existence of the grafted amine *N*-halamine on the surface of silica nanoparticles.

$$\mathbf{N\text{-}Cl + 2I^- + H^+ = N\text{-}H + I_2 + Cl^-}$$

$$\mathbf{I_2 + 2S_2O_3^{2-} = 2I^- + S_4O_6^{2-}}$$

Figure 3 : The oxidation-reduction reactions involved in the iodometric/thiosulfate titration.

Antibacterial test

It can be expected that the *N*-halamine-grafted silica nanoparticles have potent antibacterial capabilities originated from antibacterial *N*-halamine coatings. Bactericidal activity of the products was evaluated against two model bacteria *E. coli* and *S. aureus* via the modified plate counting method.

Figure 4 illustrates the antibacterial results of plate counting test. Survival bacterial colonies on the culture plates are shown as small white dots. It is clear that robust growth is found on the control plate for both *E. coli* and *S. aureus*. Obvious reduction is observed in the population of the bacterial colonies after 60 min exposure to the *N*-halamine-grafted silica nanoparticles for both two model bacteria. Only several survival colonies are seen on the culture plate, which reflect that the *N*-halamine-grafted silica nanoparticles possess excellent antimicrobial capability for killing the model bacteria.

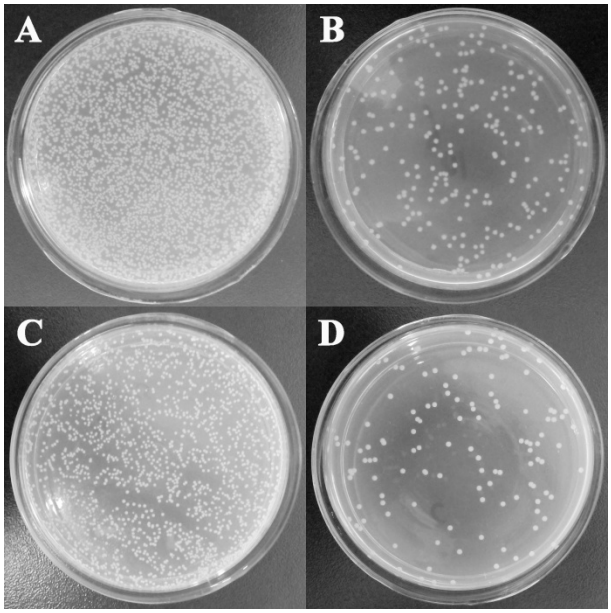

Figure 4 : Photographs showing the bacterial culture plates of *E. coli* (A and B) and *S. aureus* (C and D) upon a 60 min exposure of the control (A and C) and the *N*-halamine-grafted silica nanoparticles (B and D).

The antimicrobial activity of the *N*-halamine-grafted silica nanoparticles was further assessed using the diameter of inhibition zone (DIZ) in a disk diffusion test with *S. aureus* and *E. coli* as the model bacteria. The DIZ reflects the magnitude of the susceptibility to the model bacteria. Figure 5 displays the optical images of the DIZ result. The *N*-halamine-grafted silica nanoparticles showed a visible DIZ value of 21.2 mm for *E. coli* and 19.5 mm for *S. aureus*, indicating significant antibacterial activity against two model bacteria.

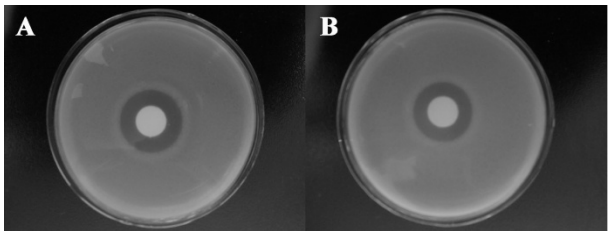

Figure 5 : Optical images of the inhibition zone against *E. coli* (A) and *S. aureus* (B) for the *N*-halamine-grafted silica nanoparticles.

CONCLUSION

In this study it was shown that the novel antibiotics *N*-halamine-grafted silica nanoparticles with core-shell structure were well fabricated by coating amine *N*-halamine on silica NPs for fighting against human pathogen bacteria. Different techniques like transmission electron microscopy, scanning electron microscopy, and the modified iodometric/thiosulfate technique were utilized to characterize the products. The plate counting method and diameter of inhibition zone tests show that the *N*-halamine-

grafted silica nanoparticles exerted superior bactericidal capability towards two model pathogenic bacteria *S. aureus* and *E. coli*.

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