Journal of Applied Polymer Science

Preparation, characterization, and antibacterial activities of quaternarized N-halamine-grafted cellulose fibers

Xiaoqin Chen, Zuliang Liu, Weiwei Cao, Chunyan Yong, Xiaodong Xing
College of Chemical Engineering, Nanjing University of Science and Technology, Nanjing 210094, China
Correspondence to: X. Xing (E-mail: xingxiaodong07@njust.edu.cn)

ABSTRACT: A viable method for coating of cellulose fiber with quaternarized N-halamine is reported in this article. The use of quaternary ammonium salt group in combination with N-halamine group can reinforce the antibacterial activity. The chemical structure of as-synthesized N-halamine precursor 4-(Bromo-acetic acid methylester)-4-ethyl-2- oxazolidinone (BEO) was characterized by ^{1}H-NMR. The cellulose fibers were characterized by Fourier transform infrared spectra and X-ray photoelectron spectra. The spectra data confirmed that the quaternarized N-halamine-grafted cellulose fibers were successfully obtained. The antibacterial properties of functional fibers were challenged with both Gram positive and Gram negative bacteria. The antibacterial tests and showed that the as-prepared antibacterial cellulose fibers exhibited powerful and rapid bactericidal performance against both Gram negative *E. coli* and Gram positive *S. aureus*. © 2015 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2015**, *132*, 42702.

KEYWORDS: biomaterials; cellulose fibers; grafting antibacterial; quaternarized N-halamine

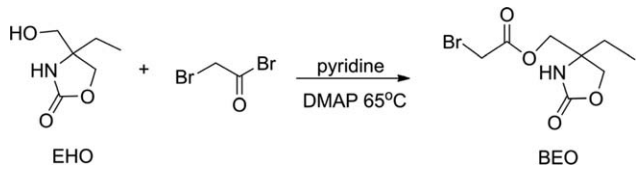
Received 30 March 2015; accepted 6 July 2015
DOI: 10.1002/app.42702

INTRODUCTION

With increasing awareness of microbial infections, extensive efforts have been conducted to prepare effective antimicrobial materials, which could be used in food packaging, hospital and dental office equipment, water purification, and household sanitation.[1–3] There are three general antibacterial strategies to control bacterial growth in solution or on material surfaces: adhesion resistance, contact killing, and biocide leaching.[4] The first approach uses non-fouling coatings to fend off bacteria from adhering onto the surface,[5] the second approach utilizes antimicrobial agents to actively kill bacteria upon contact,[6] the third approach releases active compounds to kill bacteria in solution or in the vicinity of the surface.[7,8] Since each strategy has its own advantages, it is important to develop new antibacterial strategies that combine different features into a single system to get potent and rapid antibacterial efficacy.[9–11]

N-halamines are compounds containing one or more nitrogen-halogen covalent bonds. The biocidal action of N-halamines involving direct transfer of active chlorine from the N-halamines to appropriate receptors and following oxidative reactions in the microorganism cells.[12] As one of the most effective biocides, N-halamines have high efficacies against a broad spectrum of microorganisms, including Gram negative and Gram positive bacteria, fungi, yeasts, and viruses.[13–15] Researchers have synthesized N-halamine compounds and incorporated them into common materials to prepare antibacterial N-halamine materials.[16,17] Dong *et al.*[18] designed magnetic/antibacterial bifunctional nanoparticles by immobilizing antibacterial N-halamines on modified magnetic nanoparticles. Zhang *et al.*[19] synthesized N-halamine siloxane and coated it on cotton fabrics. The as-prepared antimicrobial cotton fabrics have exhibited super strong capacity to kill harmful micro-organisms.

Quaternary ammonium salts (QAS) have many advantages over other biocides, including broad-spectrum antimicrobial activity, good environmental stability, excellent cell membrane penetrative property, and amphiphilic solubility.[20,21] The positively charged quaternary ammonium groups favor interact with negatively charged bacterial cell membrane, penetrate the cell membrane, break the balance of essential ions and induces cell lysis.[22] Therefore, as contact killing biocide, QAS have been introduced into materials such as glass, plastics and particles to provide antimicrobial functions.[23,24] However, because of the immobilization of QAS, their chain mobilities may be reduced and as a result their antimicrobial actions will be limited.[25] As a result, it is important to integrate QAS with leachable antimicrobial agents to promote their antimicrobial efficiency. Li *et al.*[26] presented two-level antibacterial coating with immobilized quaternary ammonium salts and releasable silver nanoparticles. Due to the immobilized QAS, the multilayer films retained significant antibacterial activity even after the depletion of the silver. Thus, in order to obtain powerful and fast biocidal



Scheme 1. Synthesis of 4-(bromo-acetic acid methylester)-4-ethyl-2-oxazolidinone (BEO).

capacity, it will be an operable way to incorporate N-halamine group and quaternary ammonium salt group into one molecule.

As the most abundant biomacromolecule in nature, cellulose is a cheap and nontoxic raw material.[27] Cotton as natural cellulose fiber does possess numerous –OH groups, which make it easily modified.[19] The grafting polymerization of suitable monomer onto cotton cellulose is an indispensable technique for cellulose modification without any loss in its original properties. Furthermore, with one chain end to an interface, the polymer chain produced by the "*grafting from*" approach provide a large number of functional groups. Thus, it is a significant route to combine the advantages of natural and synthetic macromolecules for a wide range of potential applications.[28]

In this study, we have synthesized N-halamine precursor (BEO) (Scheme 1) and combined it with quaternary ammonium salt group in one molecule by a simple method.[29] The modified antibacterial cotton cellulose containing immobilized quaternary ammonium salts and active chlorine was prepared in three steps. First, the dimethylaminoethyl methacrylate (DMAEMA) was grafted onto the cellulose fibers. Then, the N-halamine precursor was bonded with the grafted poly(dimethylaminoethyl methacrylate) through a quaternization reaction. Third, after a dilute household bleach treatment, the N-halamine precursor converted to amide N-halamine structure (Scheme 2) and the fibers were rendered biocidal. We supposed that the Cellulose-graft-poly(quaternarized N-halamine)(C-g-PQNH) may also possess powerful biocidal capacity. The positively charged quaternarized N-halamine groups on the cellulose fibers surface might capture negatively charged bacterial cell easily, and then kill them effectively together with active chlorine. The biocidal efficacy of the quaternarized N-halamine-grafted cellulose fibers was evaluated against Gram positive bacteria *S. aureus* and Gram negative bacteria *E. coli* respectively. Characterization of the modified cotton cellulose was also addressed in this paper.

EXPERIMENTAL

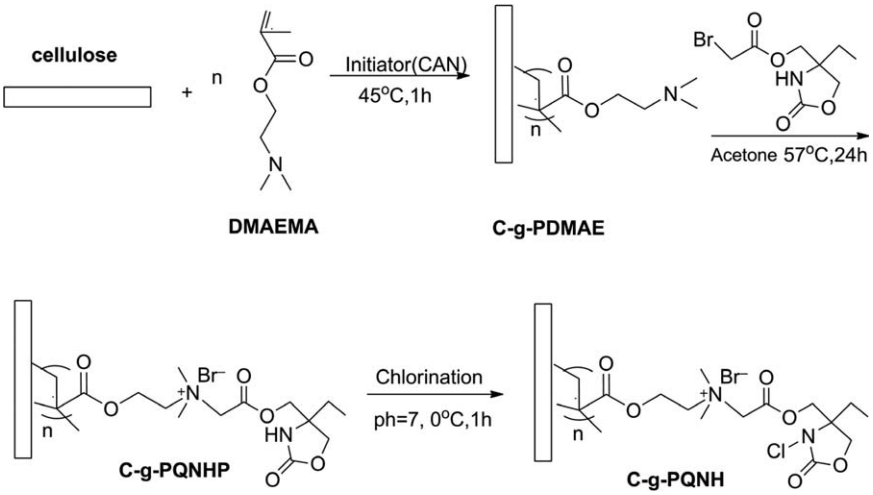
Materials and Instrumentation

Bromoacetylbromide, Dimethylaminoethyl methacrylate (DMAEMA) were purchased from Shanghai Aladdin Chemistry (China). Cotton cellulose was purchased from Shanghai DaiDi Medical Equipment (China). 4-Dimethyl-aminopyridine (DMAP) was obtained from Accela ChemBio (China). The initiator ceric ammonium nitrate (CAN) was obtained from Adamas Reagent (Basel, Switzerland). 4-ethyl-4-(hydroxymethyl)-2-Oxazolidinone (EHO) was synthesized according to a reported method.[30] Acetone, sodium hydroxide, absolute ethanol, sodium thiosulfate pentahydrate, acetic acid, pyridine, hydrochloric acid, potassium iodide, potassium dihydrogen phosphate, disodium hydrogen phosphate, sodium hypochlorite solution, and sodium chloride were purchased from Nanjing Chemical Reagent (China). All reagents were used without any purification.

^{1}H-NMR data were obtained with a Bruker AVANCE 500 MHz nuclear magnetic resonance spectrometer. The X-ray Photoelectron Spectroscopy (XPS) spectra were obtained using PHI QUANTERA II SXM X-ray photoelectron spectrometer. Fourier transform infrared (FTIR) spectra were taken on a Shimadzu IR Prestige-21 spectrometer. Scanning electron microscopy (SEM) images were obtained with a Quanta 200 field emission scanning electron microscope.

Preparation of Quaternarized N-Halamine-Grafted Cellulose Fibers

Preparation of Cellulose-graft-poly(dimethylaminoethyl methacrylate) (C-g-PDMAE). An appropriate amount of cellulose fibers were immersed in distilled water and required amount of relevant monomer DMAEMA was added, and an N_2 was purged for 10 min so as to exclude air. After adding the initiator CAN, the reaction was carried out at 55°C for 1 h in the atmosphere of N_2. After the reaction time, the modified cellulose fibers were rinsed several times by distilled water and extracted with



Scheme 2. Preparation of the quaternarized N-halamine grafted cellulose fiber.

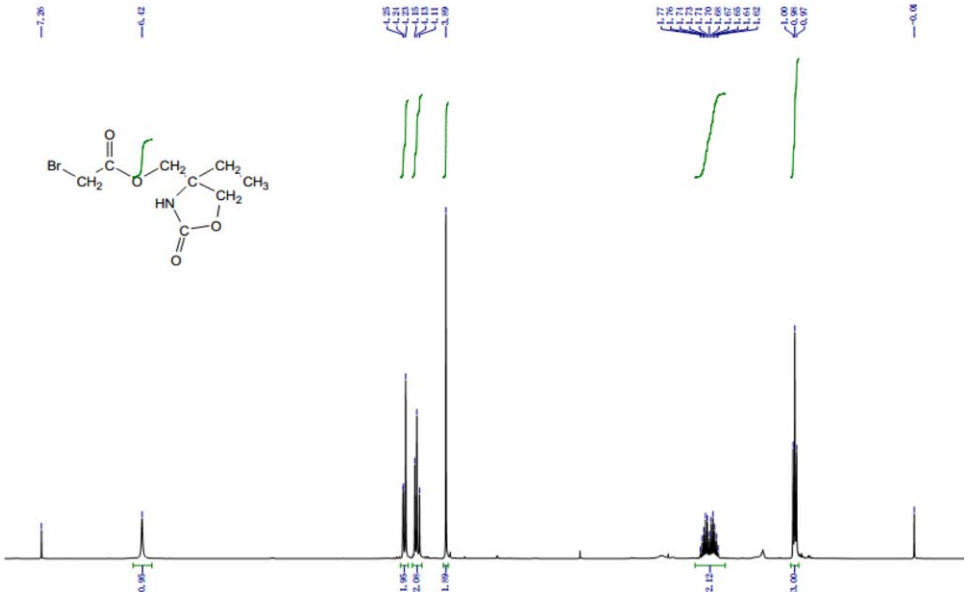


Figure 1. ^{1}H-NMR of 4-(Bromo-acetic acid methylester)-4-ethyl-2-oxazolidinone (BEO). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

acetone in a soxhlet extractor for 12 h to remove the homopolymer and unreacted monomer.

The grafting ratio (W_{gf}%) of DMAEMA-grafted cellulose fibers was calculated using the following formula.[31]

$$W_{gf}\% = \left(W_g - W_0\right)/W_0 \times 100\% \quad (1)$$

W_0 is the initial weight of cellulose fibers

W_g is the dry weight of cellulose fibers after grafting with monomer.

Preparation of Cellulose-graft-poly(quaternarized N-halamine precursor) (C-g-PQNHP)

Synthesis of 4-(bromo-acetic acid methylester)-4-ethyl-2-oxazolidinone (BEO). EHO (4.8 g, 0.033 mol), DMAP (0.01 g) and pyridine (2.66 mL, 0.033 mol) were added into 40 mL of acetone in a 100-mL flask with stirring at room temperature. When all the mixture was dissolved, 3.45 mL (0.0396 mol) of bromoacetyl bromide was dropwise added into the mixture. Then the reaction mixture was stirred vigorously at 65°C for 8 h. After 8 h reaction, the insoluble white powder was filtered out and the clear filtrate was extracted with 0.1*N* sodium hydroxide solution (3 × 50 mL) and saturated sodium chloride solution (1 × 50 mL). The solvent was removed in a rotary vacuum evaporator and the product was further purified by silica gel column chromatography. The final pale yellow oil-like product BEO was obtained after evaporation of the solvent (yield 65%). The product exhibited the following spectral data: ^{1}H-NMR (CDCl3) δ 6.42(s, 1H), 4.27–4.20(m, 2H), 4.13(t, 2H), 3.89(s, 2H), 1.81–1.56(m, 2H), 0.98(t, 3H) (Figure 1).

Appropriate amount of C-*g*-PDMAE were immersed in 10 mL acetone and excess of three times the theoretical amount of BEO was added. The samples were stirred at reflux temperature for 12 h, then removed and rinsed with acetone to remove the excess chemicals, and air-dried for further testing. The quaternization completion (W_{qc}%) was calculated using the following formula.

$$W_{qc}\% = W_1/W_2 \quad (2)$$

$$W_1 = W_q - W_g \quad (3)$$

$$W_2 = \left(W_g - W_0\right)/M_{DMAE} \times M_{BEO} \quad (4)$$

W_q is the dry weight of cellulose fibers after quaternization, W_1 is the weight increase due to quaternization,

W_2 is the theoretical weight of alkyl bromide required for complete quaternization.

Preparation of Cellulose-graft-poly(quaternarized N-halamine) (C-*g*-PQNH)

The C-*g*-PQNHP were soaked in a 10% aqueous solution of sodium hypochlorite solution (NaOCl) (0.6 wt % Cl, pH adjusted to 7 using 0.5*N* HCl solution) and stirred at 0°C for 2 h. The chlorinated cellulose fibers (C-*g*-PQNH) were washed thoroughly with distilled water to remove any free chlorine and air dried at 45°C for 1 h.[32,33]

Analytical Titration

The content of loading active chlorine in C-*g*-PQNH was determined by iodometric/thiosulfate titration method.[34,35] In this procedure, a mixture of ethanol and 0.1*N* acetic acid (9 : 1 v/v) was used as solvent. After the addition of certain amount of chlorinated cellulose fibers in the solvent, 0.1 g KI was added immediately. Two different processing methods were used. The first method, at the contact time of 60 min, the mixture was titrated with 0.0025*N* sodium thiosulfate until the yellow color disappeared at the end point. The second method, taking out cellulose fibers, and the solution was titrated with standardized sodium thiosulfate. Two samples with different grafting ratio

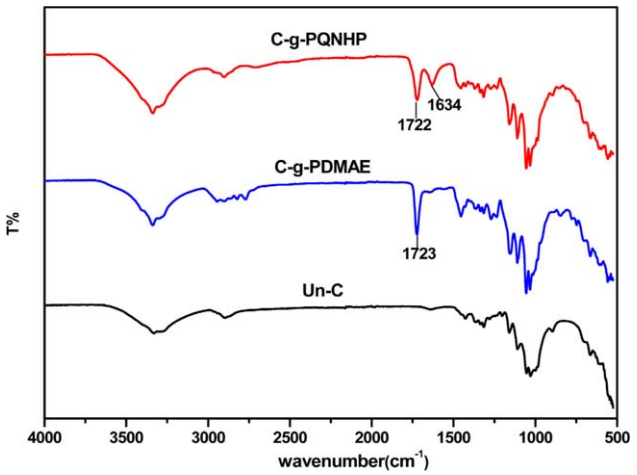


Figure 2. FTIR spectra of C-*g*-PQNHP, C-*g*-PDMAE, and Un-C. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

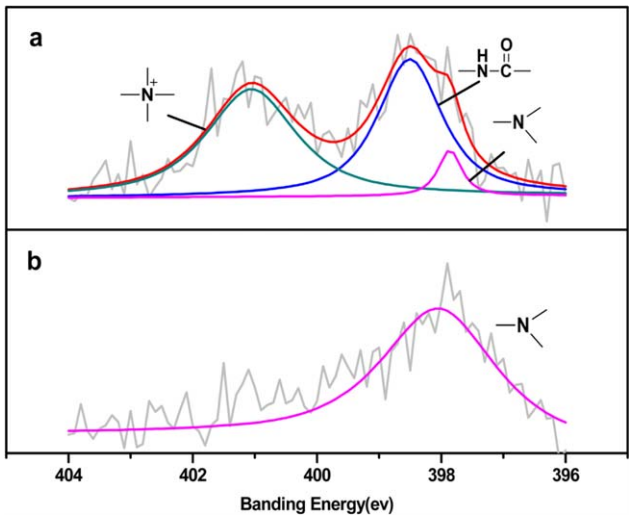


Figure 4. N 1s core-level spectra of (a) C-*g*-PQNHP and (b) C-*g*-PDMAE. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

and quaternization completion were used, and each sample would be tested for three times.

The active chlorine was calculated based on the equation:[36]

$$Cl\% = [(N \times V \times 35.45)/(2 \times W)] \times 100\% \quad (5)$$

In this equation, Cl% is the wt % of active chlorine on the fibers, N and V are the normality (equiv/L) and volume (L) of the $Na_2S_2O_3$, respectively, and W is the weight of the cellulose fibers (g).

Antibacterial Test

Antibacterial tests were conducted by kinetic test. The antibacterial properties of the two samples(C-*g*-PQNHP and C-*g*-PQNH) were challenged with Gram positive (*S. aureus*) (ATCC 6538) and Gram negative (*E. coli*) bacteria (ATCC 43895), respectively.[26] The bacteria were grown in Luria-Bertani broth at 37°C overnight, collected, washed with sterile phosphate buffered saline (PBS), and then be dispersed in PBS to a concentration of 10^5–10^6 colony forming units (CFU)/mL.

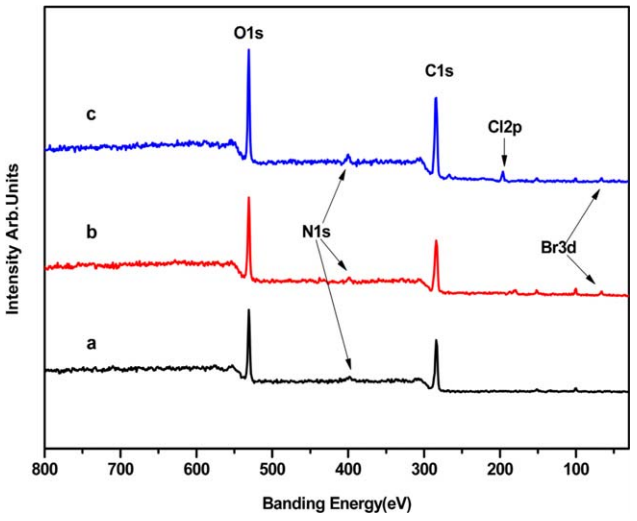


Figure 3. XPS wide scan spectra of (a) C-*g*-PDMAE, (b) C-*g*-PQNHP, and (c) C-*g*-PQNH (Cl% = 0.26%). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Totally, 0.1 g C-*g*-PQNHP were placed in test tube, then 20 mL of *S. aureus* (or *E. coli*) suspension in PBS was added. After a certain period of contact times (5, 10, 30, 45 min, and 1 h), 0.1 mL of bacteria suspension was collected and 50 μL of 0.03 wt % sodium thiosulfate was added to quench any oxidative free chlorine in the solution without affecting the growth of the bacteria. Then 0.85 mL of PBS was added to dilute the quenched solution and the solution was serially diluted, 100 μL of each dilution was placed on a LB agar plates. For the C-*g*-PQNH, both quenched solution and each dilution were placed. After incubation at 37°C for 24 h, the number of viable colony forming units (CFU) on the plate was counted for the biocidal efficacy analysis. In order to get average value, repeated tests have been performed three times with the same sample quality and bacteria concentration.

Cellulose fibers (Un-C, C-*g*-PQNHP, and C-*g*-PQNH) were placed in sterile tube contained *E. coli* for 10 min, respectively. After rinsed with deionized water, the fibers were placed in 2.5% (v/v) glutaraldehyde overnight. Then, the fibers were rinsed three times with deionized water, followed by dehydration in a series of ethanol washes and supercritical drying. The morphology of bacteria in contacted with the cellulose fibers was captured.[11]

RESULTS AND DISCUSSION

Characterization of the Grafted Cellulose Fibers

FTIR Spectra. To confirm the expected reactions, the untreated cotton cellulose (Un-C), C-*g*-PDMAE and C-*g*-PQNHP were

Table I. Different Oxidative Chlorine Titration Methods for C-*g*-PQNH

	Sample 1	Sample 2
wt % Cl 1	0.26	0.19
wt % Cl 2	0.037	0.023

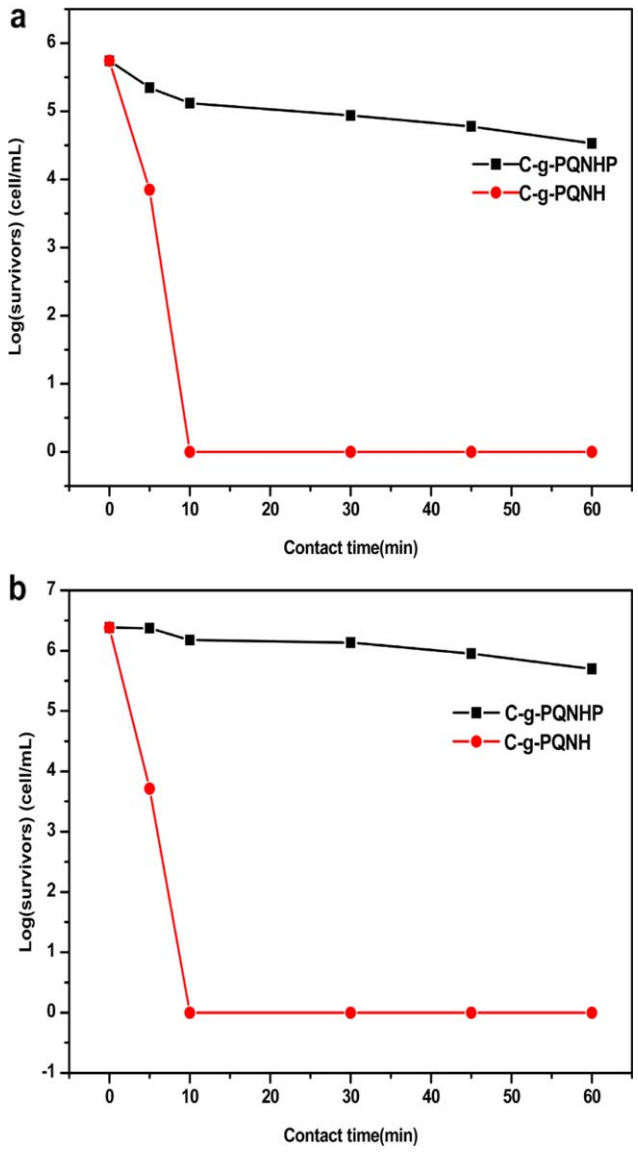


Figure 5. Antibacterial efficacies against (a) *S. aureus* and (b) *E. coli* for C-*g*-PQNHP and C-*g*-PQNH (Cl% 0.26%). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

characterized by FTIR spectroscopy. The successful immobilization of DMAEMA is verified by the introduction of an ester signal at 1722 cm^{-1}. As shown in Figure 2, for both C-*g*-PDMAE and C-*g*-PQNHP, the strong peak at 1722 cm^{-1} was assigned to the stretching vibrations of C═O group which was not observed in Un-C. For C-*g*-PQNH, a new band appeared at 1634 cm^{-1} which was assigned to the amide band I (due to C═O stretch) of BEO.[32] Therefore, the FTIR results verified that the DMAEMA had been successfully grafted onto cellulose fibers and quaternary ammonium salt had been formed after the quaternization reaction.

XPS Spectra. Detailed information about the chemical changes on cellulose surfaces were further explored by X-ray photoelectron spectroscopy (XPS) analysis. The XPS wide scans of C-*g*-PDMAE, C-*g*-PQNHP, and C-*g*-PQNH were shown in Figure 3. All of the spectrums had the main peaks of C 1s and O 1s at 283 and 531 eV, respectively. As for C-*g*-PDMAE, a new peak of N 1s appeared at about 398 eV, indicating that DMAEMA had been successfully grafted onto the cellulose fibers. In the spectrum of C-*g*-PQNHP and C-*g*-PQNH, the N 1s signal was stronger than that of C-*g*-PDMAE, signifying the increased nitrogen content. The appearance of a Br 3d signal at a binding energy of 66.5 eV verified the existence of quaternary ammonium salt groups on the fiber surface. In the case of C-*g*-PQNH, the appearance of a Cl 2p peak at a binding energy of 200 eV verified the existence of chlorine.

Further information was supported by the N 1s core-level spectra. As shown in Figure 4(b), the N 1s peak for C-*g*-PDMAE appeared at 398 eV was attributed to the nitrogen in an amino group of DMAEMA [Figure 4(b)]. As for C-*g*-PQNHP [Figure 4(a)], the N 1s core-level spectrum was curve-fitted into three peak components with binding energies at about 398.1, 398.6, and 401.0 eV. These three peaks were attributed to the amino nitrogen, amide nitrogen and positively charged nitrogen (N^+) in quaternary ammonium salt, respectively.[26] From these results, it was concluded that DMAEMA has been successful grafted onto cellulose fiber, and the quaternary ammonium salt structure has been formed.

Active Chlorine Release Measurements

The iodometric/thiosulfate titration procedure was employed to evaluate the active chlorine storage and delivery. The total content of active chlorine of the tested cellulose fibers was determined by the first method and the amount of active chlorine diffusing in the solution was determined by the second method. As can be seen from Table I, the active chlorine content in solution was very low compared with the total amount. After taking out the fibers, the Cl% decreased from 0.26% to 0.037% for sample 1 (W_{gf}% 56%; W_{qc}% 42%), and the Cl% decreased from 0.19% to 0.023% for sample 2 (W_{gf}% 40.5%;W_{qc}% 39%). This result indicated that most of the active chlorine was distributed intensively on the modified fiber surface instead of the solution.

Antibacterial Test

To fully understand the benefits of the combination system, antimicrobial efficacies of the as-prepared C-*g*-PQNHP and C-*g*-PQNH were challenged with Gram positive *S. aureus* and Gram negative *E. coli* at a concentration of 5.5×10^5 CFU/mL and 6.8×10^6 CFU/mL, respectively. The test results were presented in Figure 5.

As can be seen from Figure 5, C-*g*-PQNHP provided a 1.21 log reduction of *S. aureus* and 1.15 log reduction of *E. coli* in a contact time of 60 min. These small log reductions were possibly due to the adsorption of bacteria on the surface of the cellulose fibers. The C-*g*-PQNH inactivated both *S. aureus* and *E. coli* with log reductions of 6.83 and 5.74 within 10 min of contact, respectively. This faster and stronger antibacterial activity of C-*g*-PQNH was probably due to the enhancement of N-halamine groups and quaternary ammonium salt groups.

SEM was used to observe the morphology of the cellulose fibers after contact *E. coli* for 10 min (Un-C, C-*g*-PQNHP, and C-*g*-PQNH)

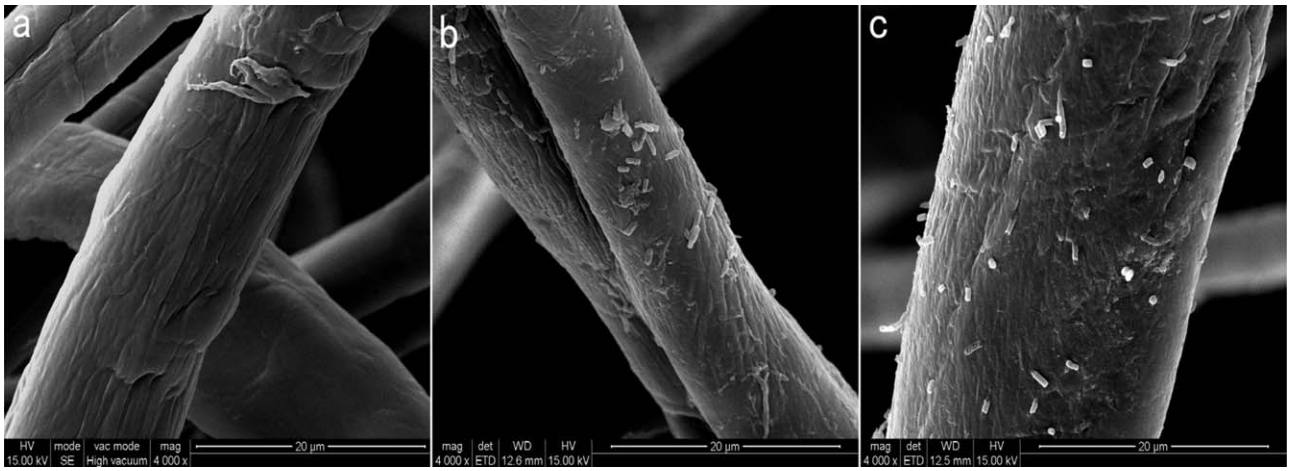


Figure 6. SEM image of cellulose fibers after contact with *E. coli* for 10 min.

[Figure 6(a-c)]. Clearly, hardly any bacteria cells could be found on the Un-C surface, it indicated that cellulose fibers itself has no bacteria adhesion ability. However, many bacteria adhering to fibers were observed on C-*g*-PQNHP and C-*g*-PQNH. The adsorption capacities of modified cellulose fibers were due to the existence of quaternary ammonium salt groups. Furthermore, most of the adsorbed bacteria were intact, while few of them were dead with damaged cell wall on C-*g*-PQNHP surface [Figure 6(b)]. Compared with C-*g*-PQNHP, there were more dead bacteria and many fragments of *E. coli* on the C-*g*-PQNH surface and no intact bacteria could be found [Figure 6(c)]. These results were consistent with kinetics of antibacterial activity, and demonstrated that quaternary ammonium groups on the fibers surface could adsorb bacteria quickly but sterilize them slowly, and in the meantime, N-halamines could damage the bacteria rapidly. By combining the advantages of quaternary ammonium groups and N-halamines, C-*g*-PQNH showed enhanced antibacterial efficacy.

CONCLUSIONS

In summary, we have prepared high efficacy antibacterial cellulose fibers coated with quaternarized N-halamine. The modified cellulose fibers could kill bacteria not only through the active chlorine but also via the immobilized quaternary ammonium salt groups. The chlorine gathered on the fiber surface could effectively kill the bacteria adsorbed by the quaternary ammonium salt groups. The antibacterial testing results also showed that the as-prepared C-*g*-PQNH exhibited stronger antimicrobial efficacies against both *E. coli* and *S. aureus* than C-*g*-PQNHP. We envisioned that the quaternarized N-halamine-grafted cellulose fibers could be potential promising material for water disinfection.

ACKNOWLEDGMENTS

This research was supported by the grant from the National Natural Science Foundation of China (No. 81130078).

REFERENCES

1. Bastarrachea, L. J.; Goddard, J. M. *J. Appl. Polym. Sci.* **2013**, *127*, 821.
2. Holtz, R. D.; Lima, B. A.; Souza Filho, A. G.; Brocchi, M.; Alves, O. L. *Nanomed.-Nanotechnol.* **2012**, *8*, 935.
3. Thomas, V.; Yallapu, M. M.; Sreedhar, B.; Bajpai, S. K. *J. Biomater. Sci. Polym. Ed.* **2009**, *20*, 2129.
4. Lichter, J. A.; Van Vliet, K. J.; Rubner, M. F. *Macromolecules* **2009**, *42*, 8573.
5. Pedron, S.; Peinado, C.; Catalina, F.; Catalina, F.; Bosch, P.; Anseth, K. S.; Abrusci, C. *J. Biomater. Sci-Polym. E.* **2012**, *23*, 1613.
6. Lu, G.; Wu, D.; Fu, R. *React. Funct. Polym.* **2007**, *67*, 355.
7. Emam, H. E.; Mowafi, S.; Mashaly, H. M.; Rehan, M. *Carbohydr. Polym.* **2014**, *110*, 148.
8. Zhang, F.; Wu, J.; Kang, D.; Zhang, H. *J. Biomater. Sci.-Polym. E.* **2013**, *24*, 1410.
9. Song, J.; Kang, H.; Lee, C.; Hwang, S. H.; Jang, J. *ACS Appl. Mater. Interfaces* **2011**, *4*, 460.
10. He, W.; Gu, X.; Liu, S. *Adv. Funct. Mater.* **2012**, *22*, 4023.
11. Hu, B.; Chen, X.; Zuo, Y.; Liu, Z.; Xing, X. *J. Appl. Polym. Sci.* **2014**, *131*, 40070.
12. Sun, X.; Cao, Z.; Sun, Y. *Ind. Eng. Chem. Res.* **2008**, *48*, 607.
13. Liu, S.; Sun, G. *Ind. Eng. Chem. Res.* **2006**, *45*, 6477.
14. Eknoian, M. W.; Putman, J. H.; Worley, S. D. *Ind. Eng. Chem. Res.* **1998**, *37*, 2873.
15. Danese, P. N. *Chem. Biol.* **2002**, *9*, 873.
16. Sun, Y.; Sun, G. *J. Appl. Polym. Sci.* **2001**, *81*, 1517.
17. Volkmann, A.; Ghosh, S. *J. Appl. Polym. Sci.* **2011**, *119*, 1646.
18. Dong, A.; Lan, S.; Huan, J. *ACS Appl. Mater. Interfaces* **2011**, *3*, 4228.
19. Zhang, B.; Jiao, Y.; Kang, Z. *Cellulose* **2013**, *20*, 3067.
20. Cheng, L.; Weir, M. D.; Xu, H. H.; Antonucci, J. M.; Kraigsley, A. M.; Lin, N. J.; Zhou, X. *Dent. Mater.* **2012**, *28*, 561.
21. Ko, C. H.; Huang, C. Y.; Hsieh, S. P.; Kuo, P. A. *Int. Biodeter. Biodegr.* **2007**, *60*, 250.

22. Roy, D.; Knapp, J. S.; Guthrie, J. T.; Perrier, S. *Biomacromolecules* **2007**, *9*, 91.
23. Saif, M. J.; Anwar, J.; Munawar, M. A. *Langmuir* **2008**, *25*, 377.
24. Gliścińska, E.; Gutarowska, B.; Brycki, B.; Krucińska, I. *J. Appl. Polym. Sci.* **2013**, *128*, 767.
25. Carpenter, A. W.; Worley, B. V.; Slomberg, D. L.; Schoenfisch, M. H. *Biomacromolecules* **2012**, *13*, 3334.
26. Li, Z.; Lee, D.; Sheng, X.; Cohen, R. E.; Rubner, M. F. *Langmuir* **2006**, *22*, 9820.
27. Klemm, D.; Heublein, B.; Fink, H. P.; Bohn, A. *Angew. Chem. Int. Edit.* **2005**, *44*, 3358.
28. Nishio, Y. Polysaccharides II; Springer: Berlin Heidelberg, **2006**; Vol. *205*, p 97.
29. Liang, J.; Chen, Y.; Ren, X.; Wu, R.; Barnes, K.; Worley, S. D. *Ind. Eng. Chem. Res.* **2007**, *20*, 6425.
30. Eknoian, M. W.; Putman, J. H.; Worley, S. D. *Ind. Eng. Chem. Res.* **1998**, *7*, 2873.
31. Ramadan, A. M.; Mosleh, S.; Gawish, S. M. *J. Appl. Polym. Sci.* **2001**, *81*, 2318.
32. Liu, S.; Sun, G. *Ind. Eng. Chem. Res.* **2008**, *48*, 613.
33. Jie, Z.; Yan, X.; Zhao, L.; Worley, S. D.; Liang, J. *RSC Adv* **2013**, *12*, 6048.
34. Chen, Z.; Sun, Y. *Macromolecules* **2005**, *38*, 8116.
35. Liang, J.; Chen, Y.; Barnes, K.; Wu, R.; Worley, S. D.; Huang, T. S. *Biomaterials* **2006**, *27*, 2495.
36. Liu, Y.; Li, J.; Cheng, X.; Ren, X.; Huang, T. S. *J. Mater. Chem. B* **2015**, *3*, 1446.