Published in final edited form as:

J Bioact Compat Polym. 2015 March ; 30(2): 157–166. doi:10.1177/0883911515569007.

Acyclic N-halamine-immobilized polyurethane: Preparation and antimicrobial and biofilm-controlling functions

Jie Luo¹, Nuala Porteous², Jiajin Lin¹, and Yuyu Sun¹

¹Department of Chemistry, University of Massachusetts Lowell, Lowell, MA, USA

²Department of Comprehensive Dentistry, University of Texas Health Science Center at San Antonio, San Antonio, TX, USA

Abstract

Hydroxyl groups were introduced onto polyurethane surfaces through 1,6-hexamethylene diisocyanate activation, followed by diethanolamine hydroxylation. Polymethacrylamide was covalently attached to the hydroxylated polyurethane through surface grafting polymerization of methacrylamide using cerium (IV) ammonium nitrate as an initiator. After bleach treatment, the amide groups of the covalently bound polymethacrylamide chains were transformed into N-halamines. The new N-halamine-immobilized polyurethane provided a total sacrifice of 10^7 – 10^8 colony forming units per milliliter of *Staphylococcus aureus* (Gram-positive bacteria), *Escherichia coli* (Gram-negative bacteria), and *Candida albicans* (fungi) within 10 min and successfully prevented bacterial and fungal biofilm formation. The antimicrobial and biofilm-controlling effects were both durable and rechargeable, pointing to great potentials of the new acyclic N-halamine-immobilized polyurethane for a broad range of related applications.

Keywords

Polyurethane; grafting; methacrylamide; N-halamine; antimicrobial; biofilm

Introduction

Polyurethane (PU) is one of the most versatile polymers for industrial, institutional, environmental, and medical applications.^{1–3} Unfortunately, in many of such applications, microbial adhesion and biofilm formation on polymer surface is a serious concern, which can damage pipes, block filters, cause spoilage of foods, and lead to device-related infections.^{4–9} As a result, there is an urgent need in the development of antimicrobial polymers, including antimicrobial PU, for a broad range of related applications. To date, a number of antimicrobial agents such as antibiotics, metal ions, quaternary ammonium salts,

© The Author(s) 2015

Reprints and permissions: sagepub.co.uk/journalsPermissions.nav

Corresponding author: Yuyu Sun, Department of Chemistry, University of Massachusetts Lowell, Lowell, MA 01854, USA. yuyu_sun@uml.edu.

Declaration of conflicting interests

The authors declare that there is no conflict of interest.

iodine, and so on have been physically mixed or covalently bonded onto PU, and some of the studies have reported encouraging antimicrobial effects.^{10–15}

Our research interests are N-halamine-based antimicrobial and biofilm-controlling polymers. N-halamines can be defined as compounds containing one or more nitrogen–halogen covalent bonds. N-halamines provide antimicrobial effects by transferring their positively charged halogens to appropriate receptors in microorganisms, leading to expiration of the microbes.^{16,17} As widely used food and water disinfectants, the antimicrobial potency of N-halamines is similar to that of hypochlorite bleach, one of the most widely used disinfectants, but N-halamines are more stable, less corrosive, and have much less tendency to generate halogenated hydrocarbons. As a result, N-halamine structures have been incorporated into various polymeric materials to achieve potent, durable, and rechargeable antimicrobial functions.^{18–23}

To expand the applications of N-halamines, here, we report a new strategy to covalently bind polymeric acyclic N-halamines onto PU through surface hydroxylation followed by methacrylamide (MAA) grafting. The amide groups in the grafted polymethacrylamide (PMAA) were transformed into N-halamines by a simple bleach treatment. The resulting N-halamine-immobilized PU demonstrated potent, durable, and rechargeable antimicrobial and biofilm-controlling functions against *Staphylococcus aureus* (Gram-positive bacteria), *Escherichia coli* (Gram-negative bacteria), and *Candida albicans* (fungi), making them attractive candidates for various applications.

Materials and methods

Materials

The polyether-based thermoplastic PU was supplied by A-dec (Newberg, OR, USA). 1,6-Hexamethylene diisocyanate (HDI), Tin(II) 2-ethylhexanoate, toluene, tetrahydrofuran (THF), and diethanolamine were purchased from Sigma–Aldrich (St. Louis, MO, USA). Cerium (IV) ammonium nitrate (CAN) and MAA were provided by VWR International, Inc. (West Chester, PA, USA). Toluene and diethanolamine were dried with 4 Å molecular sieves for 24 h before use. MAA was purified by recrystallization from distilled water. Other chemicals were used as received. The bacterial and fungal species were purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA).

Instruments

Attenuated total reflectance (ATR) infrared spectra of the samples were recorded on a Thermo Nicolet Avatar 370 Fourier transform infrared (FT-IR) spectrometer with ATR accessory (Woburn, MA, USA). X-ray photoelectron spectra (XPS) of the samples were obtained by using a PHI 5700 X-ray photoelectron spectroscopy system equipped with dual Mg X-ray source and monochromated Al X-ray source. Scanning electron microscopy (SEM) studies were performed on a LEO 1530 SEM.

Surface hydroxylation of PU (PU-OH)

PU films were obtained by solution casting. In a typical run, 2.0 g PU was dissolved in 20 mL THF at room temperature. After filtration, the PU solution was poured into a glass dish ($100 \times 15 \text{ mm}^2$) and allowed to dry in a fume hood overnight at 25 °C and 50% relative humidity (RH), followed by drying under vacuum at 40 °C for 24 h. The resulting film was uniform with a thickness around 0.15 mm.

Hydroxyl groups were introduced onto PU surface through a two-step procedure. In the first step, HDI was dissolved in toluene at a volume ratio of 1:10. PU films (2.0 g) were immersed into 50 mL of the HDI-toluene in a 250-mL three-neck round flask with the presence of 0.25 % (v/v) Tin(II) as a catalyst.²⁰ The mixture was stirred under N₂ atmosphere at 70 °C for 1 h. The isocyanate-containing PU (PU-HDI) films were taken out and washed repeatedly with toluene under sonication to remove unreacted HDI. The –NCO content on the surface of PU-HDI was determined by dibutylamine (Bu₂NH) reaction in anhydrous toluene followed by back-titration of the remaining Bu₂NH with hydrochloric acid in anhydrous ethanol using bromophenol blue as an indicator, as reported previously.²⁰

In the second step, 1.5 g PU-HDI film was immersed into 50 mL toluene containing 10 % (v/v) diethanolamine at 50 °C for 3 h under N₂ atmosphere. At the end of the reaction, the films were ultrasonically washed with ethanol to remove unreacted diethanolamine, air dried overnight, and vacuum dried at 50 °C for 24 h. In some runs, the hydroxylation process was repeated one or two more times to investigate the effects of hydroxylation on MAA grafting (see the section below).

Grafting MAA onto PU-OH (PU-MAA)

MAA was grafted onto PU-OH using CAN as an initiator.^{23,24} Briefly, 1.0 g of the PU-OH was immersed in 40 mL distilled water containing 0.04 mol/L of nitric acid and 0.004 mol/L of CAN under N₂ atmosphere. After the initiator reacted with PU-OH for 30 min, 10 wt% of MAA was added into the solution, which was stirred at 50 °C for 3 h. At the end of the reaction, the grafted PU (PU-MAA) was taken out and washed thoroughly with distilled water at 60 °C–70 °C to remove the possible homopolymers and unreacted MAA. The resulting films were vacuum dried at 50 °C for 3 days to reach constant weights.

Chlorinating the PU-MAA

To transform the amide groups of the grafted PMAA chains in PU-MAA into N-halamines, 1.0 g of the film was immersed in 30 mL diluted chlorine bleach (Clorox Company, Oakland, CA, USA) solutions containing 6000 ppm of active chlorine at ambient temperature for 1 h under constant shaking. After chlorination, the samples were washed copiously with distilled water (the washing water was tested with chlorine strips with an accuracy of 0.5 ppm of chlorine to ensure that most of the free chlorines were removed), air dried overnight, and vacuum dried to reach constant weights. The original PU films were chlorinated under the same conditions to serve as controls.

The active chlorine contents on the chlorinated PU-MAA were determined by iodometric titration.^{23,25} Briefly, 4.0 cm² of the chlorinated PU-MAA were cut into small pieces, which

were treated with 1.0 g KI in 40 mL of distilled water containing 0.05 wt% of acetic acid at room temperature under constant stirring for 60 min. The formed I_2 was titrated by 0.001 mol/L of standardized sodium thiosulfate aqueous solution. The same amount of unchlorinated PU-MAA films were titrated under the same conditions to serve as controls. The active chlorine content on the chlorinated PU-MAA was calculated according to equation (1)

$$[Cl] = \frac{35.5}{2} \times \frac{(V_{Cl} - V_0)}{S_{Cl}} \quad (1)$$

where $[Cl]$ is the active chlorine content ($\mu\text{g}/\text{cm}^2$); V_{Cl} and V_0 are the volume (mL) of sodium thiosulfate solutions consumed in the titration of the chlorinated and unchlorinated samples, respectively; and S_{Cl} is the total surface area of the chlorinated PU-MAA samples (cm^2). Each test was repeated three times, and the average was reported.

Antibacterial function

To ensure lab safety, the guidelines provided by the US Department of Health and Human Services were followed,²⁶ and appropriate personal protective equipment including gowns and gloves were used in all the microbial studies. *E. coli* (ATCC 15597, Gram-negative bacteria) and *S. aureus* (ATCC 6538, Gram-positive bacteria) were used to challenge the antibacterial functions of the N-halamine-immobilized PU. In the antibacterial tests, *E. coli* and *S. aureus* were grown in broth solutions (Luria Bertani (LB) broth for *E. coli* and tryptic soy broth for *S. aureus*) for 24 h at 37 C. The bacteria were harvested by centrifuge, washed with phosphate buffered saline (PBS), and then resuspended in PBS to densities of 10^7 – 10^8 colony forming units per milliliter (CFU/mL). A volume of 100 μL of the freshly prepared bacterial suspensions was placed between two identical chlorinated PU-MAA films ($2.0 \pm 0.1 \text{ cm}^2$). After a certain period of contact time (5–60 min), the films were transferred into 10 mL of sterilized sodium thiosulfate solution (0.03 wt%) to stop the antimicrobial actions of the films to ensure that different samples are evaluated after the same contact time. The mixture was sonicated for 5 min and vortexed for 60 s. Our preliminary studies have demonstrated that sodium thiosulfate solution at this concentration could quench the active chlorines on the films without affecting the growth of the bacteria. The suspension was then serially diluted, and 100 μL of each diluent were placed onto agar plates (LB agar for *E. coli* and tryptic soy agar for *S. aureus*). CFUs on the agar plates were counted after incubation at 37 C for 24 h. Chlorinated pure PU films were tested under the same conditions to serve as controls. Each test was repeated three times, and the shortest contact time that led to a total sacrifice of the microbial species was reported.

Antifungal function

C. albicans (ATCC 10231) was used as a representative example of fungi. In the antifungal tests, *C. albicans* was grown in yeast mold (YM) broth at 26 C for 48 h, harvested, washed, and resuspended in PBS to densities of 10^7 – 10^9 CFU/mL, as described above. A volume of 100 μL of the freshly prepared fungal suspensions were placed between two identical chlorinated PU-MAA films ($2.0 \pm 0.1 \text{ cm}^2$). After a certain period of contact time, the films

were transferred into 10 mL of sterilized sodium thiosulfate solution (0.03 wt%), sonicated for 5 min, and vortexed for 60 s. The suspension was then serially diluted, and 100 μ L of each diluent was placed onto YM agar plates. CFUs on the agar plates were counted after incubation at 26 C for 48 h. Chlorinated pure PU films were tested under the same conditions as controls. Each antifungal test was repeated three times to determine the shortest contact time for a total sacrifice of the fungi.

Biofilm-controlling function

The ability of the chlorinated PU-MAA to prevent biofilm formation was evaluated by SEM analysis. In this study, *E. coli*, *S. aureus*, and *C. albicans* were grown, harvested, and resuspended as described above. Chlorinated PU-MAA films (1.0 ± 0.1 cm²) were immersed individually in vials containing 5 mL of 10^7 – 10^8 CFU/mL of *E. coli*, *S. aureus*, or *C. albicans* suspension in PBS. The vials were shaken gently at 37 C for 30 min. After shaking, each film was taken out of the bacterial or fungal suspension with sterile forceps and gently washed three times with PBS to remove non-adherent bacteria or fungi. The films were immersed into the corresponding broth solutions and incubated at 37 C for 48 h. After incubation, the films were washed gently with PBS and fixed with 2.5% of glutaraldehyde in 0.1 M sodium cacodylate buffer at 4 C overnight. At the end of the fixation, the films were taken out and washed three times with PBS, followed by gradient dehydration with 25%, 50%, 70%, 95%, and 100% ethanol (10 min at each concentration).^{8,27} The resultant films were dried and sputter coated with gold and observed with a LEO 1530 SEM. The chlorinated pure PU films were tested under the same conditions to serve as controls.

Stability and rechargeability of the N-halamines

To evaluate the stability of the covalently bound chlorines in the N-halamines under wet conditions, a series of chlorinated PU-MAA films (1.0 cm²) were immersed in 10 mL distilled water under constant shaking (30 r/min) at ambient temperature. The water was changed weekly. The chlorine contents of the immersing water and the resulting films were titrated periodically over a 1-month period.

The stability of the N-halamine-immobilized PU was also investigated under dry-storage conditions. A series of chlorinated PU-MAA films with known chlorine contents were stored under normal lab conditions (20 C–25 C) in open air in darkness. The chlorine contents and the antibacterial and antifungal functions were tested periodically over a 6-month storage period.

To test rechargeability, the N-halamine-immobilized PU films were first treated with 0.1 M sodium thiosulfate aqueous solution at room temperature for 24 h to quench the bound chlorine and then recharged using a 10% Clorox[®] regular-bleach aqueous solution for 60 min. The chlorinated PU films were washed with distilled water, air dried, and then quenched and recharged again. After different cycles of this “quenching–recharging” treatment, the chlorine contents and antibacterial and antifungal functions of the resulting films were retested to fully evaluate rechargeability.

Results and discussion

Preparation of the N-halamine-immobilized PU

PMAA polymer chains were covalently linked to the surface of PU via HDI activation, diethanolamine hydroxylation, and CAN-initiated surface grafting polymerization of MAA monomers. The reactions were confirmed by ATR studies (Figure 1). In the spectrum of the original PU (Figure 1(a)), the bands around $3420\text{--}3200\text{ cm}^{-1}$ were attributable to N–H stretching vibrations, the bands at $3000\text{--}2800\text{ cm}^{-1}$ were due to CH_2 and CH_3 stretching, the band at 1725 cm^{-1} was caused by C = O (amide I) stretching, and the signal at 1530 cm^{-1} was caused by N–H (amide II) deforming, in good agreement with the literature data.^{20,28} After HDI activation (Figure 1(b)), in addition to the characteristic bands of PU, a new band at 2260 cm^{-1} could be detected, which was assigned to the stretching oscillation of free –NCO groups, suggesting that some HDI molecules contributed only one –NCO group to react with PU, leaving a free –NCO group on PU surface for hydroxylation with diethanolamine. Bu_2NH titration showed that the PU-HDI prepared under our conditions had $0.028\text{ }\mu\text{mol/cm}^2$ of free –NCO groups. After diethanolamine hydroxylation followed by MAA grafting (Figure 1(c)), the 2260 cm^{-1} band disappeared, indicating that the free –NCO groups were consumed in the subsequent reactions. Because of the overlap of the amide signals of the grafted PMAA with the characteristic bands of PU, little difference could be detected between spectrum of Figure 1(a) and (c).

After chlorination reactions, the amide groups of the grafted PMAA chains were transformed into acyclic N-halamines, which was confirmed by XPS studies and iodometric titration. As shown in Figure 2, in the XPS survey scans, the original PU (Figure 2(a)) and the PU-MAA (Figure 2(b)) showed peaks at 285, 403, and 533 eV, which were caused by C-1s, N-1s, and O-1s, respectively. Upon chlorine bleach treatment, some of the amide groups on the grafted PMAA side chains were transformed into N–Cl bonds. Consequently, a chlorine peak at 200 eV appeared in the XPS spectrum of the chlorinated PU-MAA (Figure 2(c)), indicating that N-halamine structures were immobilized onto PU.

Iodometric titration found that the chlorinated PU-MAA prepared under our conditions had $1.9\mu\text{g/cm}^2$ of active chlorine. Since antimicrobial and biofilm-controlling functions of the resulting N-halamine-immobilized PU could be directly affected by the active chlorine contents, various strategies were explored in our preliminary studies to control active chlorine contents on the chlorinated PU-MAA. It was found that the number of hydroxylation cycles could be used to control active chlorine contents. If the hydroxylation treatment was repeated one more time before MAA grafting and chlorination, the active chlorine contents on the resulting films could be increased to $2.6\text{ }\mu\text{g/cm}^2$. Furthermore, if the hydroxylation treatment was repeated two more times, keeping other conditions constant, the active chlorination contents could be markedly increased to $6.2\text{ }\mu\text{g/cm}^2$. These findings were not surprising: since MAA grafting polymerization was initiated by free radicals formed by the reaction of the hydroxyl groups on PU-OH with CAN,²³ more hydroxyl groups could generate more macrofree radicals on PU-OH surface to initiate MAA grafting, which could lead to higher active chlorine contents after bleach treatment.

It should be mentioned that the original PU could also be chlorinated, but the active chlorine content was much lower. After the same chlorine bleach treatment, the original PU film had $0.069 \mu\text{g}/\text{cm}^2$ of active chlorine, which disappeared after 1 week of storage in open air in darkness. These findings strongly suggested that the active chlorines on the chlorinated PU-MAA were mainly provided by the N-halamine structures of the grafted PMAA chains.

Antimicrobial and biofilm-controlling functions of the N-halamine-immobilized PU

The antimicrobial functions of the N-halamine-immobilized PU films were challenged with Gram-negative bacteria, Gram-positive bacteria, and fungi, using the chlorinated pure PU films as controls. The minimum contact time for a total sacrifice of each microbial species was determined. With $6.2 \mu\text{g}/\text{cm}^2$ active chlorine, the N-halamine-immobilized PU provided a total sacrifice of 10^7 – 10^8 CFU/mL of *E. coli*, *S. aureus*, and *C. albicans* in as short as 10 min. On the other hand, the freshly prepared chlorinated original PU films only provided less than 90% reduction of the same microbial species after as long as 8 h of contact. The potent antimicrobial activity of the N-halamine-immobilized PU films must be caused by the active chlorines, which could be transferred to the appropriate receptors in microorganisms. This effect could effectively destroy or inhibit the enzymatic or metabolic process of the species, leading to the inactivation of the bacteria and fungi.^{16,17}

The potent antimicrobial activity of the new N-halamine-immobilized PU pointed to effective biofilm-controlling functions. To evaluate this effect, Figure 3 shows the SEM results of the films in biofilm-controlling studies. After 48 h of growth in the corresponding broth solutions, a large quantity of *S. aureus* (Figure 3(a)), *E. coli* (Figure 3(b)), or *C. albicans* (Figure 3(c)) attached, aggregated, and formed layered structures on the original PU surfaces, indicating formation and development of biofilms. On the other hand, the surfaces of the chlorinated PU-MAA films were much cleaner (Figure 3(a') to (c')). Very few of scattered microbial cells could be observed, and no biofilms were formed, suggesting powerful biofilm-controlling functions of the new N-halamine-immobilized PU against Gram-positive bacteria, Gram-negative bacteria, and fungi.

Stability and rechargeability of the immobilized N-halamines

In the water-immersion studies under constant stirring, the free chlorine content in the water was less than 0.5 ppm over the 1-month storage period (the water was changed weekly), and the immersed films retained over 93% of the original covalently bound chlorines. In the dry-storage studies, under normal lab conditions in darkness, after 6 months of storage in open air, the chlorine content and the antimicrobial activities against the bacterial and fungal species were essentially unchanged. Furthermore, after five cycles of the “quenching–recharging” treatments, the chlorine contents and antimicrobial activities of the new N-halamine-immobilized PU samples were unchanged, indicating that the antimicrobial function was fully rechargeable.

Conclusion

A polymeric N-halamine precursor, PMAA, was covalently linked to PU, a versatile polymer, through surface hydroxylation followed by CAN-initiated grafting polymerization

of MAA. After a simple bleach treatment, the grafted PMAA chains were transformed into polymeric N-halamines, providing powerful, durable, and rechargeable antimicrobial activities against the tested Gram-negative bacteria, Gram-positive bacteria, and fungi. The new N-halamine-immobilized PU also prevented bacterial and fungal biofilm formation. Although more studies are needed to fully evaluate the new materials, our findings here pointed to great potentials of the N-halamine-immobilized PU as attractive candidates for a broad range of related antimicrobial and biofilm-controlling applications.

Acknowledgments

Funding

This study was sponsored by National Institute of Dental and Craniofacial Research (NIDCR) at National Institutes of Health (NIH) (grant no. R01DE018707).

References

1. Szycher, M. Szycher's handbook of polyurethanes. Boca Raton, FL: CRC Press; 1999.
2. Lelah, MD.; Cooper, SL. Polyurethanes in medicine. Boca Raton, FL: CRC Press; 1986.
3. Griesser HJ. Degradation of polyurethanes in biomedical applications—a review. *Polym Degrad Stabil.* 1991; 33:329–354.
4. Richards MJ, Edwards JR, Culver DH, et al. Nosocomial infections in medical intensive care units in the United States. *Crit Care Med.* 1999; 27:887–892. [PubMed: 10362409]
5. Hall-Stoodley L, Costerton JW, Stoodley P. Bacterial biofilms: from the natural environment to infectious diseases. *Nat Rev Microbiol.* 2004; 2:95–108. [PubMed: 15040259]
6. Van Loosdrecht MCM, Lyklema J, Norde W, et al. Influence of interfaces on microbial activity. *Microbiol Rev.* 1990; 54:75–87. [PubMed: 2181260]
7. Von Eiff C, Jansen B, Kohnen W, et al. Infections associated with medical devices: pathogenesis, management and prophylaxis. *Drugs.* 2005; 65:179–214. [PubMed: 15631541]
8. An, YH.; Friedman, RJ. Handbook of bacterial adhesion: principles, methods, and applications. Totowa, NJ: Humana Press, Inc; 2000.
9. Pavithra D, Doble M. Biofilm formation, bacterial adhesion and host response on polymeric implants—issues and prevention. *Biomed Mater.* 2008; 3:1–13.
10. Jansen B, Kohnen W. Prevention of biofilm formation by polymer modification. *J Ind Microbiol.* 1995; 15:391–396. [PubMed: 8605077]
11. Tan KT, Obendorf SK. Development of an antimicrobial microporous polyurethane membrane. *J Membrane Sci.* 2007; 289:199–209.
12. Makal U, Wood L, Ohman DE, et al. Polyurethane biocidal polymeric surface modifiers. *Biomaterials.* 2006; 27:1316–1326. [PubMed: 16181672]
13. Grapski JA, Cooper SL. Synthesis and characterization of non-leaching biocidal polyurethanes. *Biomaterials.* 2001; 22:2239–2246. [PubMed: 11456063]
14. Sun YY, Luo J, Chen ZB. Controlling biofilm formation with an N-halamine-based polymeric additive. *J Biomed Mater Res A.* 2006; 77:823–831. [PubMed: 16575910]
15. Luo J, Deng Y, Sun YY. Antimicrobial activity and biocompatibility of polyurethane—iodine complexes. *J Bioact Compat Pol.* 2010; 25:185–206.
16. Worley SD, Williams DE. Halamine water disinfectants. *Crit Rev Env Contr.* 1988; 18:133–175.
17. Elrod DB, Worley SD. Synthesis of Novel N-Halamine Biocidal Polymers. *J Bioact Compat Pol.* 1999; 14:258–269.
18. Cao Z, Sun Y. Polymeric N-halamine latex emulsions for use in antimicrobial paints. *ACS Appl Mater Interfaces.* 2009; 1:494–504. [PubMed: 20353242]
19. Yao JR, Sun YY. Preparation and characterization of polymerizable hindered amine-based antimicrobial fibrous materials. *Ind Eng Chem Res.* 2008; 47:5819–5824.

20. Sun X, Cao Z, Porteous N, et al. An N-halamine based rechargeable antimicrobial and biofilm-controlling polyurethane. *Acta Biomater.* 2012; 8:1498–1506. [PubMed: 22244984]
21. Luo J, Sun YY. Acyclic N-halamine-based biocidal tubing: preparation, characterization, and rechargeable biofilm-controlling functions. *J Biomed Mater Res A.* 2008; 84(3):631–642. [PubMed: 17635013]
22. Chen ZB, Luo J, Sun YY. Biocidal efficacy, biofilm-controlling function, and controlled release effect of chloromelamine-based bioresponsive fibrous materials. *Biomaterials.* 2007; 28:1597–1609. [PubMed: 17184837]
23. Luo J, Sun YY. Acyclic N-halamine-based fibrous materials: preparation, characterization, and biocidal functions. *J Polym Sci Pol Chem.* 2006; 44:3588–3600.
24. Bamford CH, Al-Lamee KG. Studies in polymer surface functionalization and grafting for biomedical and other applications. *Polymer.* 1994; 35:2844–2852.
25. Braun M, Sun YY. Antimicrobial polymers containing melamine derivatives. I. preparation and characterization of chloromelamine-based cellulose. *J Polym Sci Pol Chem.* 2004; 42:3818–3827.
26. Richmond, JY.; McKinney, RW. Biosafety in microbiological and biomedical laboratories. 4. Washington, DC: U.S. Government Printing Office; 1999.
27. Cen L, Neoh KG, Kang ET. Antibacterial activity of cloth functionalized with N-alkylated poly(4-vinylpyridine). *J Biomed Mater Res A.* 2004; 71:70–80. [PubMed: 15368256]
28. Guan JJ, Gao GY, Feng LX, et al. Surface photo-grafting of polyurethane with 2- hydroxyethyl acrylate for promotion of human endothelial cell adhesion and growth. *J Biomat Sci: Polym E.* 2000; 11:523–536.

Figure 1. ATR spectra of (a) the original PU film, (b) PU-HDI film, and (c) PU-MAA film. ATR: attenuated total reflectance infrared; PU: polyurethane; HDI: 1,6-hexamethylene diisocyanate; MAA: methacrylamide.

Figure 2.

XPS spectra of (a) the original PU film, (b) PU-MAA film, and (c) the chlorinated PU-MAA with 6.2 (g/cm² of active chlorine).

XPS: X-ray photoelectron spectra; PU: polyurethane; MAA: methacrylamide.

Figure 3.

SEM results of the biofilm-controlling functions of the (a) original PU film challenged with *Staphylococcus aureus*, (a') chlorinated PU-MAA film challenged with *S. aureus*, (b) original PU film challenged with *Escherichia coli*, (b') chlorinated PU-MAA film challenged with *E. coli*, (c) original PU film challenged with *Candida Albicans*, and (c') chlorinated PU-MAA film challenged with *C. albicans*.

SEM: scanning electron microscopy; PU: polyurethane; MAA: methacrylamide.

The chlorinated PU-MAA contained 6.2 (g/cm² of active chlorine).