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Antimicrobial Activity of Water Resistant Surface Coating from Catechol Conjugated Polyquaternary Amine on Versatile Substrates

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ABSTRACT: Water resistant polymer with adhesive behavior is reported to exhibit long-term antimicrobial activities under diverse conditions. To obtain highly adhesive polymer with prolonged antimicrobial activities and catechol moiety, 2-chloro-3′,4′-dihydroxyacetophenone (CCDP) has been quaternized to poly(dimethylaminoethyl methacrylate) (PDMA). Later, for gaining the antimicrobial effects, the polymer (CCDP-q-PDMA) was quaternized with 1-bromododecane (C12) [CCDP/C12-q-PDMA]. X-ray photoelectron spectroscopy rectified the quaternization and adhesion of the polymer onto different substrates. The CCDP/C12-q-PDMA coated substrates exhibited remarkable bacterial killing efficiency against both Gram-positive *Staphylococcus aureus* and Gram-negative *Escherichia coli*, after incubation for 24 h. The remarkable antimicrobial activities exhibited in aqueous medium at 60°C for up to 60 days indicate the strong adhesiveness of CCDP/C12-q-PDMA on the substrate. Therefore, the synthesized polymer offers merit as a potential coating material, to protect a broad range of material from microbial contamination, for a prolonged period of time, under a highly stressed water environment. © 2014 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2014**, *131*, 40708.

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INTRODUCTION

At present, contamination of the surfaces of various organic and inorganic materials by different kinds of microorganisms has been a major global issue in different fields like medical devices, healthcare products, water purification systems, hospitals, dental office equipment, food packaging, household sanitation and so forth.[1–4] The surfaces act as reservoir for the deposition of various microbes, by forming a biofilm, which results in the destruction of the material itself as well as the source of spreading infections.[5] To avoid this adverse situation, a bioactive surface is highly desirable, which can put off bacterial contamination. Strategies have been taken under consideration, by preparing surfaces with antibiotics, surfactants, triclosan and so on.[6–9] The immobilization of antimicrobial peptides is being exhaustively investigated.[10,11] Although these mentioned materials have some great advantages, the potential local toxicity, susceptibility to proteolysis, pH sensitivity, sensitization and allergy after repeated applications, and the cost of synthesis represent the main disadvantages.[12–14] To date, a number of antimicrobial polymers have been reported, and some studies have achieved very promising results, behind which the "permanent immobilization via coating of these polymers onto the surface" is the favored argument.[15,16] But shortcomings still remain, regarding the coating procedures, adhesive properties of the coating materials, and antimicrobial properties of the materials after coating and so forth.[17] Another drawback of the reported antimicrobial polymer is the lack of resistance to stability in aqueous media. So, substrates coated with this type of polymer are not protected from microbial attack, when they are applied in an aqueous environment.[18] As an adhesive material, catechol is regarded as being responsible for impressive adhesive properties.[19–21] The finding has stimulated great interest in exploiting catechol for improving interfacial adhesion on different materials. Under alkaline condition, a quinone form of catechol is formed through self-polymerization with the accompanied oxidation of catechol groups, and is able to show

adherent properties on many substrates.[22,23] The oxidized quinone form of catechol further allows it to participate in reactions with various functional groups, including thiol, amine, and quinone itself, via Michael addition or Schiff base reaction, to produce covalently grafted functional layers.[22,23]

The goal of this study is the development of a water resistant antimicrobial polymer, having the ability to coat a wide range of substrates with antimicrobial activity, following a simple and convenient method. The catechol and hydrophobic alkyl group have been conjugated to poly(dimethylaminoethyl methacrylate) (PDMA), following quaternization, to be a highly adhesive material that can be applied on a broad range of substrates. The polymer was well characterized by ^{1}H-NMR and Fourier transform infrared (FTIR), and X-ray photoelectron spectroscopy (XPS) investigation was applied to characterize the polymer, as well to rectify the successful coating on substrate. The antibacterial effect of polymer coated substrates was carried out against Gram-positive *Staphylococcus aureus* and Gram-negative *Escherichia coli*, which was shown to offer highly effective and stable antimicrobial activity under wet process.

EXPERIMENTAL

Materials

2-Mercaptoethanol, dimethylaminoethyl methacrylate (DMA), tetrahydrofuran, 2-chloro-3′,4′-dihydroxyacetophenone (CCDP), ethanol, 1-bromododecane (C12), trizma base (99%, Sigma), trizma HCl (99%, Sigma), diethyl ether, hexane, *S. aureus*, *E. coil*, MRS, LB, ager, and d-chloroform ($CDCl_3$) reagent were purchased from Sigma Aldrich Reagent Company.

Synthesis of (2-chloro-3′,4′-dihydroxyacetophenone/1-bromododecane) Quaternized Poly(dimethylaminoethyl methacrylate) (CCDP/C12-q-PDMA)

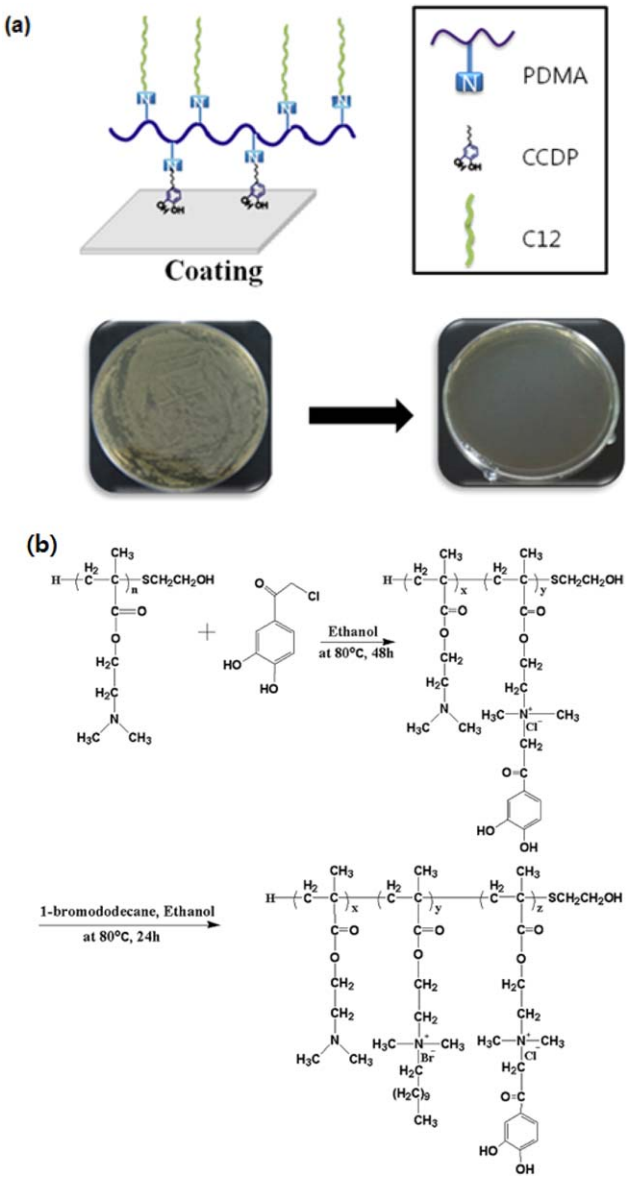
PDMA terminated with a hydroxyl group at one end was prepared by chain transfer polymerization, using 2-mercaptoethanol as a chain transfer agent. The PDMA (0.1 mmol) and 2-chloro-3′,4′-dihydroxyacetophenone (CCDP, 1.5 mol) was dissolved in 50 mL of anhydrous ethanol in a 250-mL flask, following our previous report of preparing CCDP-q-PDMA.[21] The mixture was stirred for 48 h at 70°C. After stirring, the solvent was evaporated in a rotary evaporator. Diethyl ether was added in the polymer, to form precipitation. The resulting CCDP quaternized PDMA (CCDP-q-PDMA) was dried in vacuum and analyzed. The yield of the product was 75%.

Finally, CCDP-q-PDMA (0.1 mmol) and 1-bromododecane (8.5 mol) were dissolved in 80 mL of anhydrous ethanol in a 250-mL flask. The mixture was stirred for 24 h at 80°C. After stirring, the solvent was evaporated, using a rotary evaporator. Diethyl ether was added in the polymer, to form precipitation. The resulting CCDP/C12-q-PDMA was dried in vacuum and analyzed. The yield of the product was 80%.

^{1}H NMR (400 MHz, D_2O, δ): 3.9–4.1 (2H, OCH_2), 2.3–2.6 (2H, CH_2), 0.6–0.9 (3H or 2H, CH_3, or CH_2), 0.9–1.1 (18H, $(CH_2)_9$), 1.8–2.3 (3H, NCH_3), 3.9–4.1 (2H, CH_2), 6.8–7.4 (1H, CH).

Surface Modification by CCDP/C12-q-PDMA

Surface modification using CCDP/C12-q-PDMA was performed, by immersing substrates in a buffer solution of (10 mM Tris, pH 8.5), methanol with 10 mg/ mL of (catechol/C12) quaternized PDMA at room temperature. After 24 h of incubation, the coated substrates were rinsed extensively with deionized water, and dried under a stream of argon, for further experiments.



Scheme 1. Illustration of (a) preparation of surface coated substrates under alkali condition and (b) the synthesis of CCDP/C12-q-PDMA. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Characterization

CCDP/C12-q-PDMA was characterized by ^{1}H-NMR (Bruker AVANCE 400 spectrometer operating at 400 MHz), using d-chloroform as a solvent. FTIR (Thermo scientific, Nicolet 380) spectrometer was also used to characterize the synthesis CCDP/C12-q-PDMA. The spectra, including the background scan, were collected at a resolution of 4 cm^{-1}, and 128 scans were averaged. XPS spectra were obtained to measure the surface

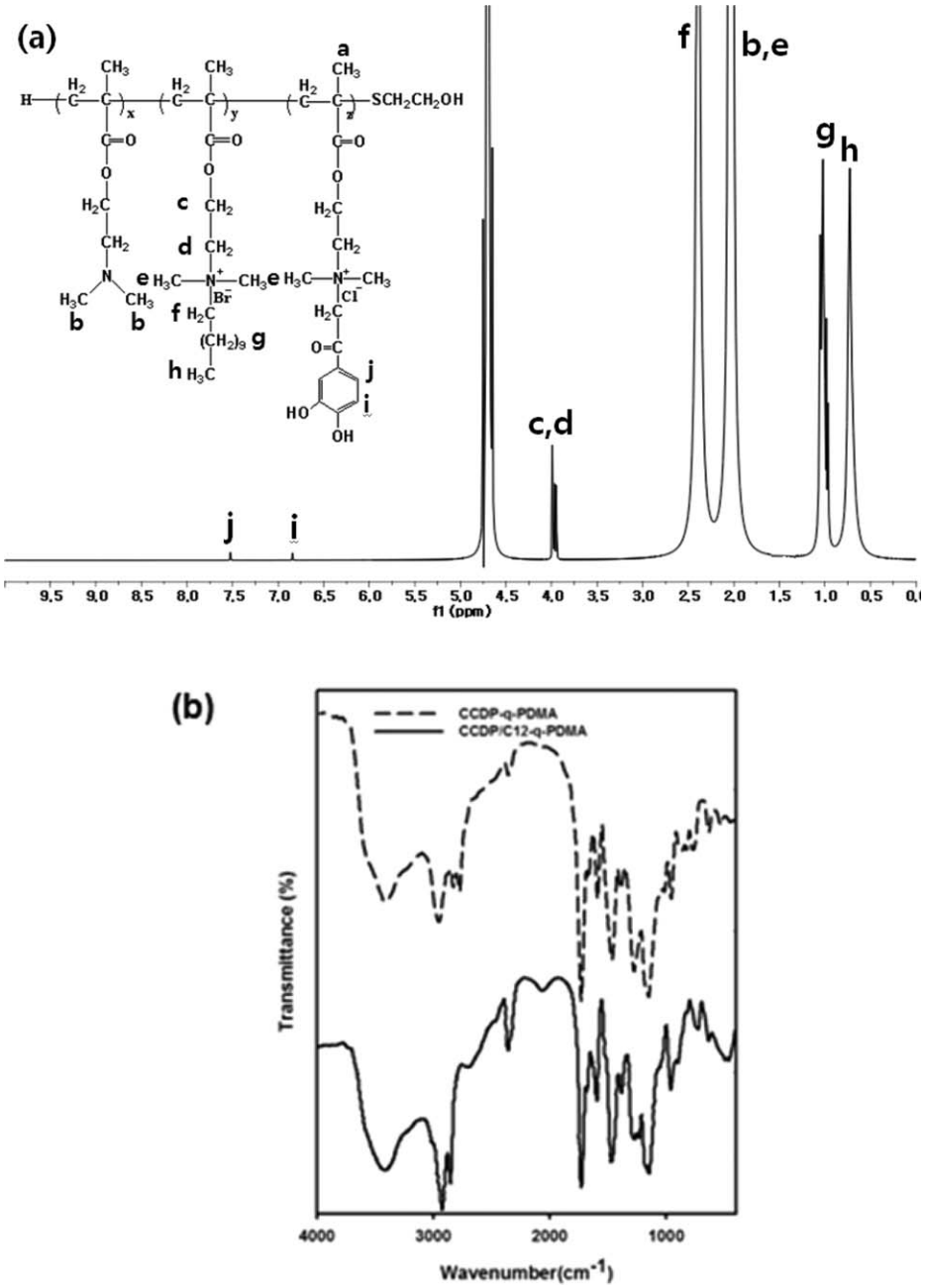


Figure 1. (a)[1]H-NMR spectrum of CCDP/C12-q-PDMA and (b) FTIR spectra of CCDP-q-PDMA and CCDP/C12-q-PDMA.

atomic composition, using an Omicrometer ESCALAB (Omicrometer, Taunusstein, Germany), with amono-chromated Al KR (1486.8 eV) 300 W X-ray source, under ultra high vacuum ($<10^{-9}$ Torr). The takeoff angle was fixed at 45°, except as otherwise mentioned, and all spectra were calibrated, using the hydrocarbon C 1s peak (284.5 eV).

Antibacterial Activity

The antibacterial activity of the CCDP/C12-q-PDMA on solid substrates against Gram-positive bacteria *S. aureus* (American Type Culture Collection (ATCC) 25923) and Gram-negative bacteria *E. coli* (ATCC 12435) were assessed by a viable cell counting method, following our previous reports.[21,24] A freeze-dried ampoule of *S. aureus* and *E. coli* was opened, and the cultures were carried out on bacterial nutrient agar plate by using wire loop, and incubated for 24 h at 37°C. After 24 h incubation, broth cultures of *S. aureus* and *E. coli* were prepared by inoculating one colony into 10-mL sterilized nutrient broth, which was then incubated at 37°C overnight, under shaking at 150 rpm. The composition of 1 L of nutrient broth, for *S. aureus*, was 10.0 g of peptone, 5.0 g of NaCl, 5.0 g of beef extract (MRS broth) at pH 7 and for *E. coli*, was 10.0 g of bactotryptone, 5.0 g of yeast extract, 10 g of NaCl (Luria-Bertani); 20.0 g of agar powder was added, to prepare solid nutrient agar.

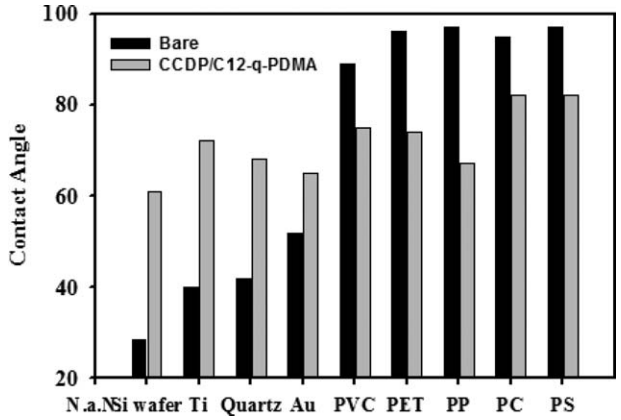


Figure 2. Contact angle investigation on different substrates after coating with CCDP/C12-q-PDMA.

Table I. Investigation of the Thickness of the Surfaces After Coating with CCDP-q-PDMA and CCDP/C12-q-PDMA by Elipsometer

Sample	Thickness (Å) without $Nalo_4$	Thickness (Å) with $Nalo_4$
CCDP-q-PDMA	12.10 ± 5.64	13.64 ± 3.08
CCDP/C12-q-PDMA	6.22 ± 4.76	12.05 ± 3.04

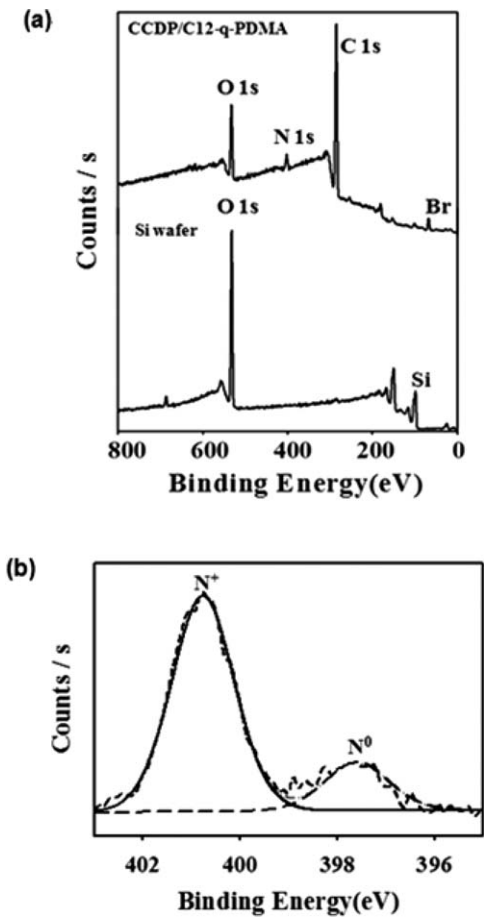


Figure 3. XPS survey spectrum of CCDP/C12-q-PDMA. (a) Wide-scan XPS spectra and (b) narrow scan of N1s spectra.

Overnight cultures were measured by UV-vis spectroscopy at 600 nm, with absorbance adjusted to 0.6 to confirm the turbidity standard according to the McFarland scale, and at this stage the cultured contained about approximately 10^8 cells/mL. Then, the microorganism suspensions were diluted, and the suspension used for the tests contained 10^5 to 10^6 colony forming units (CFU). For evaluation of antimicrobial activity, the bacterial (*S. aureus*, *E. coli*) suspension having a concentration of 10^6/mL was sprayed using a chromatographic sprayer on the prepared slides. Slides were dried in air and placed in Petri dish, and growth agar was added. The Petri dishes were sealed and further incubated at 37°C for 24 h. An uncoated slide was used as standard, and the number of viable colonies grown was used as reference.[25] For long-term antimicrobial activities of CCDP/C12-q-PDMA, the coated substrate was immersed in water in a Petri dish, and incubated at 25 and 60°C for up to 60 days. After the desired time interval, the PP films were collected, and the antimicrobial activity was investigated, following the method mentioned earlier. The investigated coated PP films were washed properly, immersed in water again, and incubated, following the same condition.

Fluorescence Microscopy

A LIVE/DEAD BacLight bacterial viability kit (L-7007, Invitrogen, Carlsbad, CA) was used to determine the bacterial cell viability. CCDP/C12-q-PDMA coated PP surfaces were prepared by the same procedure as described earlier, except that PP surfaces with an original size of 2.0 × 2.0 cm^2 were used. A solution of mixed SYTO 9 and PI dyes was prepared according to the

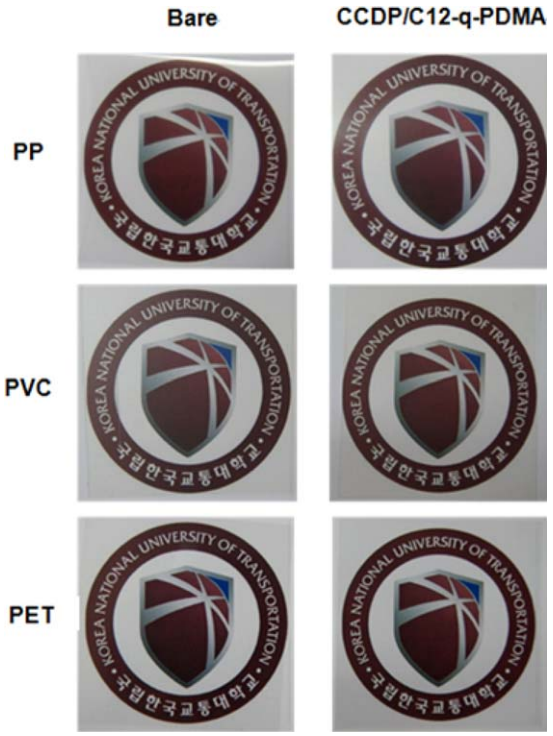


Figure 4. Optical images to show the transparency of CCDP/C12-q-PDMA before and after coating on PP, PVC, and PET surfaces. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

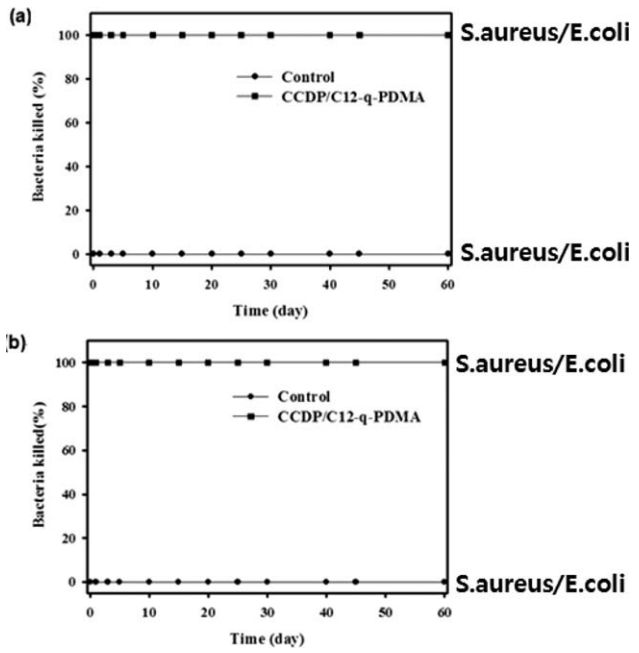


Figure 5. Bacterial cell killing efficiency (%) of CCDP/C12-q-PDMA coated PP film under highly stressed as a function of time (a) at 25°C, and (b) at 60°C against Gram positive (*S. aureus*) and Gram negative (*E. coil*).

manufacturer's instructions. The bacterial suspension (1 mL, 5 × 10^5 CFU/mL) was mixed with fluorescent dyes (10 μL) for 15 min. This bacterial suspension with the dyes (10 μL) was dropped onto CCDP/C12-q-PDMA coated PP surfaces or untreated PP surfaces (control), and a glass coverslip (2.0 × 2.0 cm^2) was placed on the droplet. This slide sample was incubated with shaking at 200 rpm for 1 h at 37°C. The slide was also placed in the dark to avoid the bleaching of the fluorescent dyes by ambient light. The slide was examined using a fluorescence microscope (LSM 510, Carl-Zeiss). Images were obtained using an oil immersion 20 objective lens.

RESULTS AND DISCUSSION

Polymeric materials play a fascinating role, with the potentiality to assist the researcher in searching for a better way to develop antimicrobial polymer that can easily bind to the surfaces of the matter.[21,26] But when substrates coated with antimicrobial polymer come into contact with aqueous medium, the long-term antimicrobial efficiency is difficult to gain. Therefore, their short time activity results in the demand to search for a way to achieve their long-term activity. The strategy can be considered as developing a polymer having highly anchoring properties, under aqueous medium, onto a wide range of substrates accompanied with antimicrobial attributes. In the construction of preparing surface coated substrates, we have synthesized a polymer showing both adhesive and antimicrobial properties, where we have first prepared PDMA. PDMA was synthesized, using 2-mercaptoethanol as a chain transfer agent, and AIBN as an initiator, following radical polymerization.[24,27] To show adhesiveness of the polymer, we integrated 2-chloro-3′,4′-dihydroxyacetophenone (CCDP) into the prepared PDMA, following quaternization, and to achieve antimicrobial effects, further quaternization of the remaining DMA segment was allowed, using long chain 1-bromododecane (CCDP/C12-q-PDMA).[21] The overall synthesis routes of CCDP/C12-q-PDMA are presented in Scheme 1(b). First, catechol moiety (CCDP) was quaternized with PDMA, to get CCDP-q-PDMA. Figure 1(a) ^{1}H-NMR investigation indicates that the degree of quaternization was estimated to be 15 catechols per PDMA backbone, derived from comparing the relative integration of the methyl proton of PDMA at 1.8–2.3 ppm and the aromatic protons of CCDP at 6.8–7.4 ppm. As a consequence, to finally achieve the antimicrobial property, the existing remains of grafted PDMA were further quaternized, followed with 1-bromododecane (C12). The ^{1}H-NMR peak at 0.9–1.1 ppm was attributed to the methylene proton (18H, $(CH_2)_9$) of 1-bromododecane, where 85 units of 1-bromododecane was quaternized with PDMA in Figure 1(b) FTIR spectroscopy was further performed, to

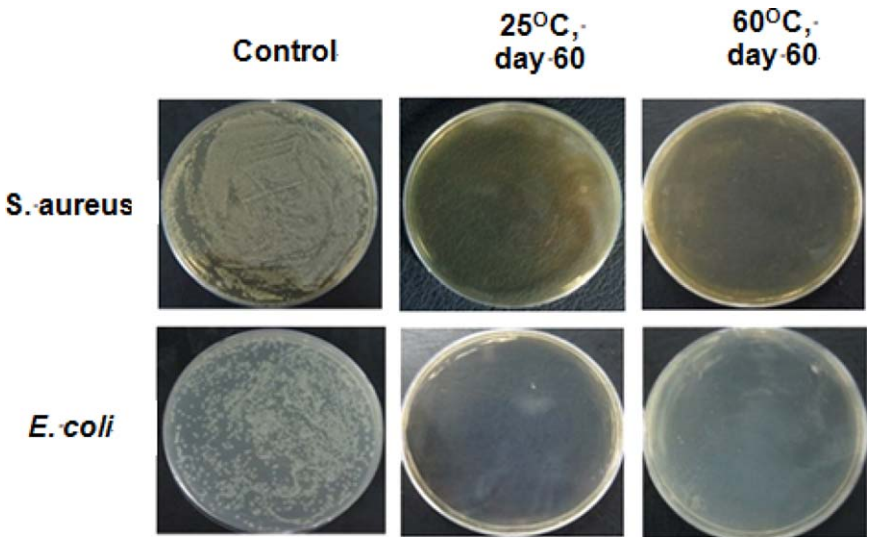


Figure 6. Photographs of colonies with CCDP/C12-q-PDMA coated on PP film and untreated PP film against Gram positive (*S. aureus*) and Gram negative (*E. coil*). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

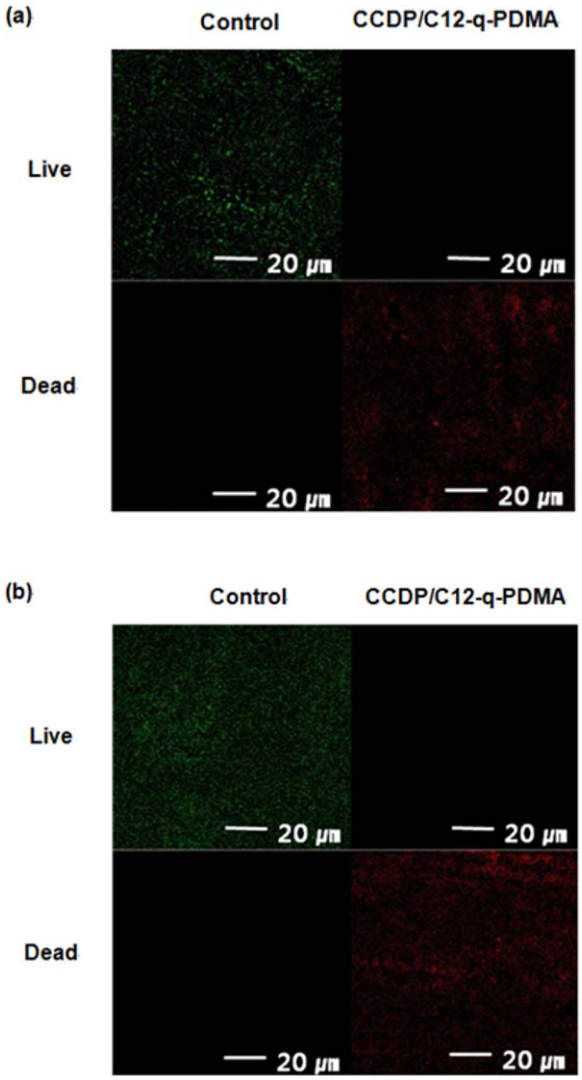


Figure 7. Fluorescence microscopy images of bacteria untreated [(a) *S. aureus*; (b) *E. coli*] and treated with CCDP/C12-q-PDMA [(a) *S. aureus*; (b) *E. coli*] for 1.0 h after staining with SYTO 9 and PI. The scale bar is 20 μm. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

characterize the CCDP-q-PDMA and CCDP/C12-q-PDMA. Figure 1(b) shows the FTIR spectrum of the polymers, where the absorption bands at 1510 cm^{-1} confirmed the presence of the ring stretching from CCDP, and the peak at 2921 cm^{-1} indicates the presence of alkyl chain of 1-bromododecane in the PDMA.[21]

To verify the adhesive properties of CCDP/C12-q-PDMA as coating material, we selected different type of substrates. All the investigated substrates were washed properly, and coating on the surfaces was performed, by dipping in CCDP/C12-q-PDMA solutions. Before characterization, all coated substrates were dried at 45°C for 24 h. The significance of a coating material is strongly dependent on its surface properties, like wettability, morphology and so forth.[28] Taking this into consideration, contact angle examination was conducted. From Figure 2, it is seen that the contact angle was significantly increased, compared to the bare surfaces of Si wafer, titanium, quartz, and Au; whereas it was decreased, when the polymer was used as a coating material on the polymer surfaces of polyvinyl chloride (PVC), polyethylene terephthalate (PET), PP, polycarbonate (PC), and polystyrene, which was indicated by the adhesiveness of the synthesized polymer. To strongly support the adhesiveness, we investigated the thickness of the substrates, using elipsometer before and after using $NaIO_4$. A change in thickness has been found, when CCDP/C12-q-PDMA was used as the coating material. Before adding $NaIO_4$, the thickness was around 6.22 Å; whereas, it became double after the addition of $NaIO_4$, as in Table I. The increased thickness clearly indicates the adhesion of the CCDP/C12-q-PDMA polymer onto the surfaces. Figure 3(a) presents the wide scan spectrum, showing the presence of nitrogen and bromine. Figure 3(b) reports the narrow scale XPS spectra related to nitrogen, where it is seen that there are two peaks at 397.2 and 401 eV, corresponding to the quaternary and tertiary nitrogen of PDMA.[21] The XPS results rectify the successful quaternization of PDMA by 1-bromododecane.

Transparency of coating material is a highly attractive feature, and strongly demanded in various fields. Considering this attribute, the optical properties of CCDP/C12-q-PDMA coated PP, PVC, and PET were conducted. Figure 4 shows a clear, well visible insight of the surfaces of all applied materials, indicating the significant transparent property of CCDP/C12-q-PDMA.

Finally, the ability of the coating materials to prevent microbial contamination was conducted, by measuring the bacterial cell killing efficiency on CCDP/C12-q-PDMA coated PP surface, against Gram-positive *S. aureus* and Gram-negative *E. coli,* after 24 h. The properties of CCDP/C12-q-PDMA, which showed 100% killing efficiency against both type of bacteria, indicates high antimicrobial activity by CCDP/C12-q-PDMA. Figure 5 represents quantitative data of the investigated antimicrobial activity of CCDP/C12-q-PDMA. The main goal of our work is to prepare a highly adhesive coating agent, showing antimicrobial activities under high stress condition in water, for a prolonged period of time. As a corollary, antibacterial assessment against both types of bacteria on the surface of CCDP/C12-q-PDMA coated on PP film was repeatedly examined at 25 [Figure 5(a)] and 60°C [Figure 5(b)], for 60 days. The coated PP films were immersed in water, and on different days they were collected, to evaluate the antimicrobial activity. Figure 5(a,b) indicate the highly efficient antimicrobial properties, with 100% killing efficiency. This result indicates two potential attributes of CCDP/C12-q-PDMA: one is the highly adhesive behavior of CCDP/C12-q-PDMA in aqueous medium, due to the anchoring behavior of catechol; and the other is the long-term antimicrobial activity of 1-bromododecane from CCDP/C12-q-PDMA. As shown in Figure 6, after 24 h of incubation, dense bacterial colonies were observed on the control MRS agar for *S. aureus* and LB agar for both *E. coli*. However, the MRS and LB agar plates with the CCDP/C12-q-PDMA coated PP film exhibited no growth of bacterial colonies, indicating excellent antibacterial activity. The findings from antibacterial investigations indicate that materials can be protected, after using CCDPC12-q-PDMA as a coating material, enabling their durability for long period

of time, without acting as a source of any contamination to the environment.

The viability of bacterial cells on the surface was further examined by staining, using the bacterial LIVE/DEAD fluorescent dyes. The effects of treating Gram-negative and Gram-positive bacteria with CCDP/C12-q-PDMA are shown in Figure 7. For the uncoated PP surface as control, most bacterial cells fluoresced green, indicating that these cells were viable. The fluorescence microscopy images show that the cell viability, in the case of the control samples, is clearly seen by green fluorescence and the cells treated with the compound show complete membrane permeabilization, as indicated by red fluorescence in Figure 7(a,b) *S. aureus* and *E. coli*, respectively. This shows that the bacteria were not able to adhere strongly onto these surfaces, which is advantageous for the surface to retain antimicrobial activity.

CONCLUSIONS

In conclusions, we have successfully synthesized water resistant polymer, CCDP/C12-q-PDMA. The developed CCDP/C12-q-PDMA exhibited extreme anchoring properties onto a wide range of substrates. Antibacterial killing efficiency investigation, against both Gram-positive and Gram-negative investigated bacteria, show that it can be applied to achieve prolonged antimicrobial activity in water under diverse circumstances, which can protect the substrates from microbial contamination.

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REFERENCES

1. Padmanabhuni, Z.; Luo, J.; Cao, Z.; Sun, Y. *Ind. Eng. Chem. Res.* **2012**, *51*, 5148.
2. Neely, A. N.; Maley, M. P. *Clin. J. Microbiol.* **2000**, *38*, 724.
3. Larson, E.; Olmsted, R. N. *Am. J. Infect. Control* **2001**, *29*, 69.
4. Kang, C. K.; Lee, Y. S. *J. Ind. Eng. Chem.* **2012**, *18*, 1670.
5. Hall-Stoodley, L.; Costerton, J. W.; Stoodley, P. *Nat. Rev. Microbiol.* **2004**, *2*, 95.
6. Han, H.; Wu, J.; Avery, C. W.; Mizutani, M.; Jiang, X.; Kamigaito, M.; Chen, Z.; Kuroda, K. *Langmuir* **2011**, *27*, 4010.
7. Zurita, R.; Puiggali, J.; Rodriguez-Galan, A. *Macromol. Biosci.* **2006**, *6*, 58.
8. Ruggeri, V.; Francolini, I.; Donelli, G.; Piozzi, A. *J. Biomed. Mater. Res. A* **2007**, *81*, 287.
9. Tiller, J. C.; Sprich, C.; Hartmann, L. *J. Control. Release.* **2005**, *103*, 355.
10. Fulmer, P. A.; Lundin, J. G.; Wynne, J. H. *ACS Appl. Mater. Interfaces* **2010**, *2*, 1266.
11. Perron, G. G.; Zasloff, M.; Bell, G. *Proc. Biol. Sci.* **2006**, *273*, 251.
12. Marr, A. K.; Gooderham, W. J.; Hancock, R. E. *Curr. Opin. Pharmacol.* **2006**, *6*, 468.
13. Park, Y. J.; Park, S. Y.; In, I. *J. Ind. Eng. Chem.* **2012**, *18*, 231.
14. Li, Y.; Kumar, K. N.; Dabkowski, J. M.; Corrigan, M.; Scott, R. W.; Nusslein, K.; Tew, G. N. *Langmuir* **2012**, *28*, 12134.
15. Ilker, M. F.; Nusslein, K.; Tew, G. N.; Coughlin, E. B. *J. Am. Chem. Soc.* **2004**, *126*, 15870.
16. Ishitsuka, Y.; Arnt, L.; Majewski, J.; Frey, S.; Ratajczek, M.; Kjaer, K.; Tew, G. N.; Lee, K. Y. *J. Am. Chem. Soc.* **2006**, *128*, 13123.
17. Sambhy, V.; Peterson, B. R.; Sen, A. *Langmuir* **2008**, *24*, 7549.
18. Chakrabarty, S.; King, A.; Kurt, P.; Zhang, W.; Dennis, E. O.; Lynn, F. W.; Lovelace, C.; Rao, R.; Kenneth, J. W. *Biomacromolecules* **2011**, *12*, 757.
19. Nahain, A. A.; Lee, S. Y.; In, I.; Lee, K. D.; Park, S. Y. *Int. J. Pharm.* **2013**, *450*, 208.
20. Oh, Y. J.; Hwan, C.; Lee, H.; Park, K.; Lee, H.; Park, S. Y. *Chem Commun.* **2012**, *48*, 11895.
21. Nam, J. A.; Nahain, A. A.; Kim, S. M.; In, I.; Park, S. Y. *Acta Biomater.* **2013**, *9*, 7996.
22. Waite, J. H.; Tanzer, M. L. *Science* **1981**, *212*, 1038.
23. Dalsin, J. L.; Hu, B. H.; Lee, B. P.; Messersmith, P. B. *J. Am. Chem. Soc.* **2003**, *125*, 4253.
24. Ding, Z.; Chen, G.; Hoffman, A. S. *Bioconjug. Chem.* **1996**, *7*, 121.
25. Chen, S.; Chem, S.; Jiang, S.; Xiong, M.; Luo, J.; Tang, J.; Ge, Z. *ACS Appl. Mater. Interfaces* **2011**, *3*, 1154.
26. Mosaiab, T.; Jeong, C. J.; Shin, G. J.; Choi, K. H.; Lee, S. K.; Lee, I. S.; In, I.; Park, S. Y. *Mater. Sci. Eng. C* **2013**, *33*, 3786.
27. Nahain, A. A.; Lee, K. S.; Mosaiab, T.; Park, S. Y. *J. Appl. Polym. Sci.* **2013**, *130*, 168.
28. Colak, S.; Tew, G. N. *Biomacromolecules* **2012**, *13*, 1233.