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# Scalable and Rechargeable Antimicrobial Coating for Food Safety Applications

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Supporting Information

**ABSTRACT:** Environmental surfaces are common avenues for microbial contamination and transmission in food-processing establishments. We recently synthesized a polymer that combines both *N*-halamine and dopamine functional groups to form a novel antimicrobial coating material. A series of chemical (titration) and biological ("sandwich" and anti-inhabitation) tests were designed to prove the stability and functionality of as-developed coating material. Halamine−dopamine polymer-coated stainless-steel surface inactivated 6 $\log_{10}$ CFU of both *Staphylococcus aureus* and *Escherichia coli* O157:H7 under experimental detection limit within 10 min of contact time. After three "discharge−recharge" cycles, the surface maintained the same antimicrobial effectiveness; 60% of the surface chlorine remained after 10 "discharge−recharge" cycles. In addition, the coating thickness and chlorine content could be further tuned through adjusting the formulation of the coating. We also demonstrated that this coating material could be easily scaled up to apply on real food equipment parts through a spray-coating method. Thus our polymer material has great potential to produce a high-performance, low-cost, and easy-to-apply coating on food-associated environmental surfaces for food safety preventive-control applications.

**KEYWORDS:** *N-halamine, dopamine, antimicrobial coatings, food safety*

## INTRODUCTION

Foodborne illness is a major burden to both public health and the profitability of the food industry in the United States. The U.S. Center for Disease Control and Prevention (CDC) estimates that each year in the United States foodborne diseases result in about 48 million sicknesses, 128 000 hospitalizations, and 3000 deaths.[1] The total economic loss was estimated as $15.6 billion by the U.S. Department of Agriculture (USDA).[2] The majority (>91%) of current foodborne illnesses are caused by consuming foods contaminated with harmful microorganisms such as fungi, bacteria, virus, and so on.[1] Foodborne pathogens also contribute significantly to the increasing food recalls in the food industry: Almost half of these recalls are due to microbiological contamination.[3] On average, about $10 million direct costs are associated with a single recall of a food product, not including brand damage and lost sales.[3,4] There is therefore a need for novel strategies to reduce microbial contamination of food products.

Microbial contamination can occur at multiple stages of the food production process including production itself, processing, distribution, and preparation.[5−9] Because bacteria (e.g., *Pseudomonas*, *Listeria monocytogenes*, *Salmonella*, etc.) can attach and colonize on solid surfaces, if not eliminated in a timely manner, they can quickly proliferate and form biofilms.[10] Mature biofilms are resistant to common sanitation treatments, including aggressive sanitizing/cleaning agents, and are thus notoriously difficult to remove.[11] The bacteria embedded in the biofilm are a potential source of cross-contamination for any food product that comes in contact with the afflicted surface during processing.[12−14] In addition, surfaces that do not have direct contact with food, including machinery parts, floors, ceilings, walls, and sewage systems, can also introduce contamination into food products via indirect routes.[6] To avoid such contamination, it is important to continuously prevent bacterial attachment and growth, not simply kill bacteria during sanitation procedures.

In recent years, there have been several efforts to develop novel surface-sanitizing technologies such as electrolyzed water[15] or a combination with physical treatment such as ultrasound.[16] However, these methods provide a reactive method rather than a proactive method for removing biofilm and preventing microbial cross-contamination on hard surfaces. Antimicrobial coatings provide one means to continuously resist bacterial contamination of surfaces and thus can be used as a preventive control method. Antimicrobial coatings for food equipment have been extensively reviewed by Bastarrachea et al.[17] However, very few of the technologies developed to date can be easily translatable from the laboratory

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to the food industry in the near future due to the high cost of materials, compromised biocidal functions in the food-associated environment, or challenges in the process of scaling up. For instance, silver-based antimicrobial coating has been commercially available; however, it is limited by high cost and ineffectiveness in the food manufacturing environment due to fouling from organic load and antimicrobial resistance.[18,19] In recent years, *N*-halamine antimicrobial chemistry has attracted a great deal of interest due to its potent and broad biocidal function, low cost, and low toxicity.[20] *N*-Halamines are a group of compounds that contain a nitrogen−halogen bond formed through the halogenation of a nitrogen−hydrogen bond, and chlorine-based *N*-halamine is the most widely researched. The antimicrobial mechanism of *N*-halamine is similar to that of other chlorine-based compounds such as hypochlorite; however, the difference is that the chlorine is fixed in the molecule through covalent bonding.[16] Previously, *N*-halamines have been intensively researched for broad applications including water treatments, textiles, biomedical devices, and so on.[17,20,21] There have been some previous reports of *N*-halamine coatings on materials that are commonly used in food manufacturing such as stainless steel,[22,23] plastics,[24,25] paints,[26] and so on. However, the fabrication methods used in these previous studies are either high-cost or require complex surface pretreatments, lengthy and costly coating procedures, or cost-prohibitive quantities of *N*-halamine polymers. In this study, we focused on incorporating *N*-halamine polymers into low-cost and easy-to-apply coating materials.

The most immediate challenge when developing coatings for hard surfaces is ensuring sufficient adhesion. Previous research on *N*-halamine coatings typically employed methods such as grafting that required complex surface treatments, corrosive/toxic agents, or harsh and long-time heating to achieve stable adhesion to surfaces.[22−24] In this study, we took advantage of mussel-inspired dopamine chemistry.[27] Dopamine provides strong adhesion to diverse surfaces even in a wet environment[28,29] and is relatively safe for biological applications.[30] In addition, dopamine-functionalized polymers also have the dual function of cross-linking with either amine- or thiol-terminated molecules to form a thick and stable network.[30−32] In this study, we designed a polymer structure that combines both *N*-halamine antimicrobial and dopamine adhesive functionalities. We demonstrated that the halamine−dopamine polymer-coated stainless-steel surface completely inactivated more than 6 $\log_{10}$ CFU of both Gram-positive and Gram-negative bacteria within 10 min of contact time. Furthermore, even after three "discharge−recharge" cycles, the chlorine content remained sufficiently high to maintain a similar bacteria-killing effectiveness. The coating thickness and chlorine content could be tuned by adjusting the formulation of the coating. Finally, we developed a scalable and convenient spray-coating method to apply the coating to real food equipment parts. This new antimicrobial material may find broad applications as high-performance, low-cost, and easy-to-apply coatings, especially for food-associated environmental surfaces.

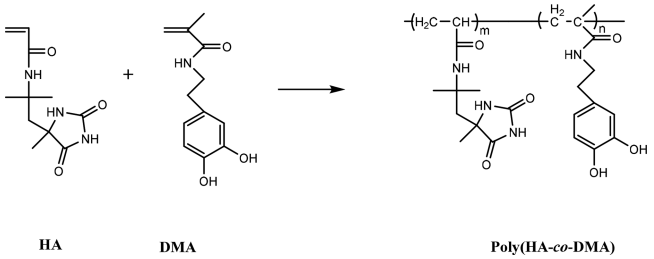
## MATERIALS AND METHODS

**Materials.** *N*-(1,1-Dimethyl-3-oxobutyl)acrylamide was purchased from TCI Chemical, Japan. 3,4-Dihydroxyphenethylamine hydrochloride, 2,2′-azobis(2-methylpropionitrile) (AIBN), and polyethylenimine (PEI) were purchased from Sigma-Aldrich (St. Louis, MO). Polypropylene, high-density polyethylene, and stainless-steel 316L were purchased from McMaster-Carr (Aurora, OH). A bacterial LIVE/DEAD kit (Invitrogen) was purchased from Life Technologies Corporation (Eugene, OR). All chemicals and reagents were used as received. Bacterial strains *Staphylococcus aureus* ATCC 3359 (isolated from a hospital) and *Escherichia coli* O157:H7 ATCC 43890 (isolated from human feces) were obtained from the Food Microbiology Lab at Cornell University, Ithaca, NY.

**Chemical Synthesis.** *N*-Halamine precursor vinyl monomer hydantoin acrylamide (HA) [*N*-(2-methyl-1-(4-methyl-2,5-dioxoimidazolidin-4-yl)propan-2-yl)acrylamide] was synthesized following a previously reported method based on the Bucherer-Bergs reaction.[26] Dopamine vinyl monomer dopamine methacrylamide (DMA) was synthesized following a previous method described by Lee et al.[27] $^1$H NMR (proton nuclear magnetic resonance) was employed to confirm the structures of synthesized monomers. Detailed descriptions about the synthesis procedures can be found in the Supporting Information.

A free-radical polymerization method was used to prepare the halamine−dopamine copolymers (Scheme 1). For polymerization, 100% MeOH (20 mL) was used as the solvent, HA (7 mmol) and DMA (3 mmol) were used as monomers, and AIBN (2,2′-azobis(2-methylpropionitrile)) (40 mg) was used as an initiator. The reaction mixture was bubbled with nitrogen gas for 20 min to remove oxygen. The polymerization occurred at 60 °C for 3 h. After cooling to room temperature, a white sticky polymer precipitated out in the bottom of the flask and was collected. The polymer was further washed using hot MeOH. The wet polymer product was then dissolved in 80% EtOH at 100 mg/mL as a stock solution and stored in the refrigerator. The polymers were characterized with $^1$H NMR, FT-IR (Fourier transform infrared spectroscopy, Bruker Vertex V80V vacuum FT-IR system), and GPC (gel permeation chromatography, Waters ambient temperature GPC/DMF).

**Scheme 1. Synthesis of Halamine−Dopamine Copolymers**




**Dip-Coating and *N*-Halamine Activation.** A dip-coating method was used to coat halamine−dopamine polymer on various substrates, and chlorination was used to activate the *N*-halamine moieties (Scheme 2). A working solution of halamine−dopamine polymer (poly1, HA-*co*-DMA, HA/DMA 7:3) at 2 mg/mL was prepared from the stock solution using 80% EtOH as the solvent. To test the coating adhesion, five different material substrates including 316L stainless steel, plastics (PP, PVC, HDPE), and glasses were cut into 1 in$^2$ coupons and cleaned with DI water. Next, the coupons were immersed in the coating solution for 3 h. After that, the coupons were briefly washed with 80% EtOH to remove any nonbonded or loosely attached polymer molecules and dried in a fume hood for at least 2 h. The poly1-coated coupons were chlorinated through treatment with 10% bleach solution (8.25% hypochlorite, pH adjusted to 7.0 with HCl, and available chlorine content ~4000 ppm) for 30 min, washed thoroughly with DI water to remove any free chlorines on the surface, and dried overnight in a fume hood. The coatings were characterized with contact-angle goniometer (ramé-hart) and XPS (X-ray photoelectron spectroscopy).

**Surface Chlorine Titration.** The immobilized oxidative chlorine content (*N*-halamine) on the surface of halamine−dopamine polymer-coated coupons was determined using an iodometric/thiosulfate titration method.[25] In brief, two coupons (1 in$^2$) were put into a flask containing potassium iodine (KI), water, and HCl, and the coupons were stirred at room temperature for 10 min. Next, the resulting solution was added to 0.5% starch solution and titrated by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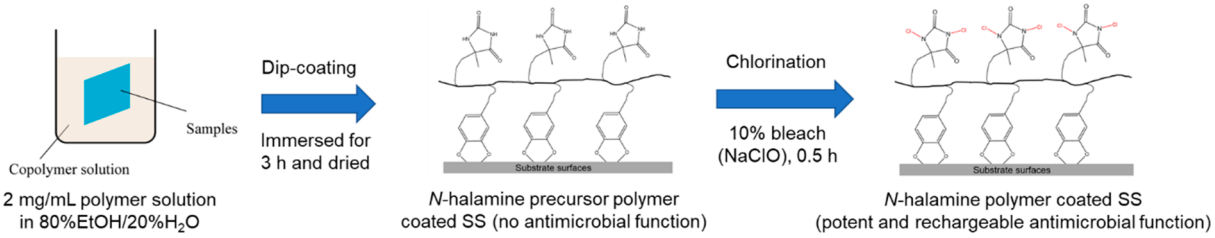
**Scheme 2. Dip Coating and Chlorination**




2 mg/mL polymer solution in 80%EtOH/20%$H_2O$

*N*-halamine precursor polymer coated SS (no antimicrobial function)

*N*-halamine polymer coated SS (potent and rechargeable antimicrobial function)

sodium thiosulfate. The oxidative chlorine content was calculated using the following formula: $[Cl^+]$ (atoms/cm$^2$) = $(N \times V)/2A \times 6.02 \times 10^{23}$ mol$^{-1}$, where $N$ and $V$ are the normality (equiv·L$^{-1}$) and volume (L) of the titrant sodium thiosulfate and $A$ is the total surface area of titrated sample (cm$^2$). Three groups were repeated for coupons of each material.

To investigate the coating stability and rechargeability, stainless-steel samples were used for titration. After first chlorination and titration (R0), the samples were retrieved, washed thoroughly with DI water, and chlorinated under the same condition as previously described, and this process was defined as one "discharge−recharge" cycle (R1). This process was repeated for an additional nine times, and the resulting stainless-steel (SS) coupons were designated as R10.

**Surface Biocidal Efficacy Test.** The biocidal efficacy of halamine−dopamine-coated stainless-steel surfaces was determined using a "sandwich" contact-kill testing method, as previously described.[25] A Gram-negative bacterium of *E. coli* O157:H7 and a Gram-positive bacterium of *S. aureus* were used in this study. A single colony of each bacteria was transferred to 15 mL of brain heart infusion (BHI) broth and incubated at 37 °C for 16 h. The culture was pelleted through centrifugation, washed twice with Butterfield's phosphate buffer (BPB), and finally resuspended in BPB buffer. The bacterial population density was estimated by the $O.D._{640\ nm}$, and an inoculum was prepared. A 25 μL aliquot of the inoculum (~$4 \times 10^7$ CFU/mL bacteria) was added to the center of the square coupon, a second identical coupon was placed on the sample, and the sandwich was compressed with a sterile weight to ensure complete contact with inoculated bacteria. At the contact times of 10, 30, and 60 min, the coupons were transferred to 5 mL of $Na_2S_2O_3$ solution (0.05 N) and vigorously vortexed to quench any oxidative chlorine residuals and to detach surviving bacteria from the sample. Ten-fold serial dilutions were made for all samples, and each dilution was plated on trypticase soy agar plates. The plates were incubated at 37 °C for 48 h, and bacterial colonies were enumerated and recorded for biocidal efficacy analysis. The number of bacteria (CFU/sample) of inoculum and the number of bacteria at each contact time were calculated as shown here: bacterial number ($\log_{10}$CFU/sample) = $\log_{10}$[(bacterial counts on agar plate × dilution factor × 5 mL)/(0.025 mL)].

The rechargeable antimicrobial function was determined using the same "sandwich" method, but only Gram-positive bacteria *S. aureus* were inoculated. After the first antimicrobial test cycle (R0), all samples were retrieved and sanitized with 70% EtOH. Because all chlorines on the *N*-halamine-coated SS surface were quenched during the antimicrobial testing procedure (vortexing in sodium thiosulfate solution), the coating was totally "discharged" and had no antimicrobial activity. The coating was "recharged" by repeating the same chlorination procedure. This "recharged" *N*-halamine-coated SS (R1) was used for the second "sandwich" test following the same procedures. The same procedure was repeated one more time, and the result was recorded as R2.

**Bacterial Inhabitation Prevention Test.** To investigate the comprehensive antimicrobial control effect of coated surfaces under organic load, a bacterial inhabitation prevention test was performed according to a previously published method with some modifications.[17] In brief, the overnight culture of *S. aureus* was prepared to make a final cell density of $10^6$ CFU/mL in LB (Luria−Bertani) medium. Next, triplicate samples (1.5 × 0.5 cm$^2$) were immersed in the bacterial solution for 3 h at room temperature. The samples were then taken out, rinsed with PBS buffer, and stained with a bacterial LIVE/DEAD kit. The samples were observed via fluorescence microscopy (EVOS FL Cell Imaging System, Thermo Scientific) to assess bacterial viability. The wavelengths for live/dead imaging were green fluorescent protein (470/22 nm) for live cells and red fluorescent protein (531/40 nm) for dead cells. Only bacteria that both attached and survived on the surface emitted green fluorescence (green dots with diameter between 1 and 5 μm) on the surface. The density of living bacterial remaining on the surface of bare stainless steel (SS-Ctrl), *N*-halamine precursor polymer-coated (SS-Poly1, unchlorinated), and *N*-halamine polymer-coated (SS-Poly1-Cl, chlorinated) was compared.

**Tuning Coating Thickness through Cross-Linking.** To further increase the thickness of coating, PEI was used as a cross-linking agent, as previously described.[27] In brief, 2 mg/mL of polymer solution was prepared with 80% EtOH and 20% Tris-HCl buffer (pH 8.5), with the final pH of the solution around pH 8.0. PEI (100 mg/mL) solution was prepared in DI water. The polymer solution and PEI solution were mixed together with a molar ratio of Poly1/PEI 1:5. Then, the solution was used for the dip-coating procedure: The samples were immersed in the solution for 10 s, taken out, and dried/cured for 1 h under fume hood. Afterward, samples were washed thoroughly with 80% EtOH to remove any loosely attached molecules. Then, the samples were chlorinated under the same chlorination conditions as those described above. The samples were characterized via water contact angle, FT-IR, and XPS. In addition, a spray coating method was performed for poly1 solution both with and without PEI using a mist sprayer bottle (samples were oriented vertically during spraying). The coated samples were dried/cured, cleaned, and chlorinated with the same method as dip coating. All of these dip-coated and spray-coated samples were titrated for chlorine content.

**Scale-Up and Application on Real Food Equipment.** The optimized formulation of poly1+PEI was used for coating on an actual food equipment part through spray coating. A stainless-steel (304) pipe was taken directly from a food-processing plant and cleaned thoroughly with soap and water. PEI-formulated poly1 solution (5 mg/mL) was sprayed on the external surface using a mist sprayer and was totally dry within 5 min. After further curing in air for 1 h, both coated and uncoated pipe surfaces were sprayed with 0.5% bleach and dried. The chlorination process was repeated three times to ensure sufficient chlorination. After that, the whole pipe was rinsed thoroughly with DI water to remove any free chlorine residues on the surface. To visualize oxidative chlorines on *N*-halamine-coated pipe, a simple method based on the mechanism of iodometric titration was used. An indicator solution containing water, acid, potassium iodine, and starch solution was prepared in situ, and a drop of this solution was dripped on the surface. If enough oxidative chlorines were immobilized on the surface, then the solution would turn from colorless to blue within 5 s. If no immobilized oxidative chlorines were on the surface, then the solution would stay colorless for at least 1 min. A swabbing method was also developed in which a cotton Q-tip was dipped into the indicator solution and briefly swabbed on the surface. If the surface was successfully coated with *N*-halamine coating, then the cotton changed from colorless to blue color within 5 s. However, if there was no coating or the *N*-halamine was not activated, then cotton stayed colorless for at least 1 min.



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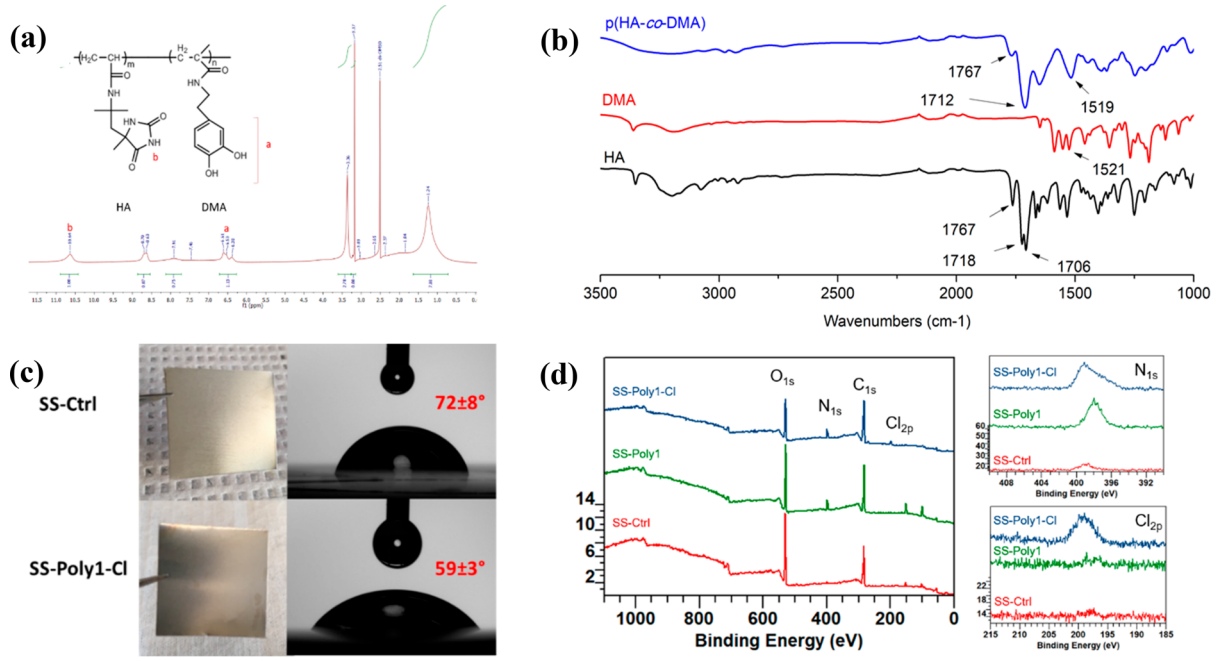


**Figure 1.** Characterization of polymer and coating: (a) $^{1}$H NMR spectrum of synthesized p(HA-*co*-DMA). HA/DMA was calculated as 7:3. (b) FT-IR spectra of HA, DMA, and p(HA-*co*-DMA). (c) Appearances and contact angles of bare stainless steel (SS-Ctrl) and polymer-coated and chlorinated stainless steel (SS-Poly1-Cl). (d) XPS spectra of C, N, and Cl in bare stainless steel (SS-Ctrl), poly1-coated and unchlorinated stainless steel (SS-Poly1), and poly1-coated and chlorinated stainless steel (SS-Poly1-Cl). Poly1: p(HA-co-DMA).

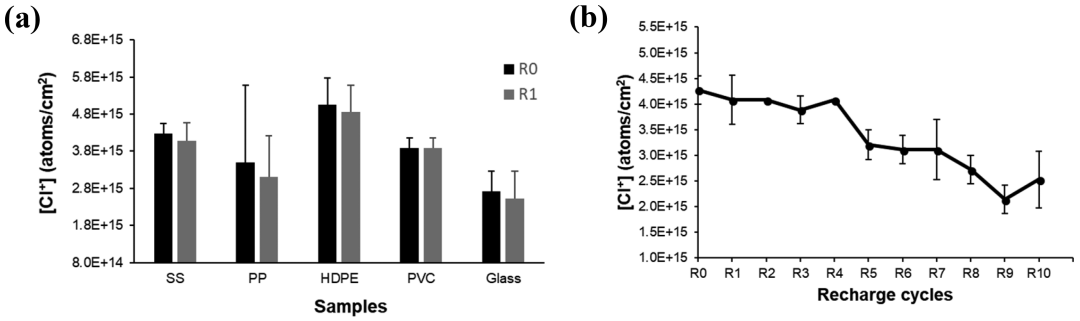


**Figure 2.** Comparison of oxidative chlorine content on: (a) different materials: SS (stainless steel), PP (polypropylene), HDPE (high-density polyethylene), PVC (polyvinylchloride), and glass and (b) polymer-coated stainless steel after different "recharge−discharge" cycles. For all experiments, $N = 3$. "R" represents recharge cycle. R0: coated and chlorinated. R1: after 1 "discharge−recharge" cycle. R10: after 10 "discharge−recharge" cycles.

The antimicrobial control effect of the halamine−dopamine coating on the SS pipe was performed according to a previously reported method with some modifications.[19] In brief, overnight-cultured bacteria (*S. aureus*) were centrifuged, washed with PBS, and resuspended in PBS (phosphate-buffered saline) to prepare a bacterial inoculum with cell density of $5 \times 10^8$ CFU/mL. A clean and sterile cotton swab was dipped into the bacteria solution and evenly spread on a surface area of 1.5 in$^2$ for both coated and uncoated regions. After 1 h of contact, the surviving bacteria on the surface were recovered by swabbing thoroughly with a cotton swab premoistened with BPW (buffered peptone water) solution. The cotton swab was then put into 1 mL of BPW solution and vortexed for 2 min to detach all bacteria into the solution. After that, the solution was serial diluted and plated in TSA (trypticase soy agar) plates. The plates were incubated at 37 °C for 24 h, and bacterial colony-forming units (CFUs) were recorded and compared for evaluating the antimicrobial control effect. For both coated and uncoated surfaces, triplicate areas were tested in each experiment. After the first experiment (Experiment 1), the pipe was sanitized thoroughly with 70% EtOH and washed thoroughly with a light duty scrub sponge (Scotch-Brite, 3M) using tap water. Then, the surface was treated with 0.5% chlorine bleach, dried, rinsed thoroughly, and stored overnight for the next experiment (Experiment 2). This procedure was repeated three times in total on three different days, and the results were recorded as Experiments 1, 2, and 3, respectively. The results of triplicate experiments were combined. The significance of difference ($P < 0.05$) in the number of *S. aureus* that survived on the coated and uncoated surfaces was determined with ANOVA and a general linear model using SPSS for Windows 7.

## RESULTS AND DISCUSSION

The halamine−dopamine copolymer p(HA-*co*-DMA) was characterized through NMR and FT-IR. In the NMR spectrum (Figure 1a), the characteristic peaks of both HA ($\delta$ 10.7, N−H) and DMA ($\delta$ 6.25 to 6.75, catechol) were observed in the polymer chain, yet the double bond in each monomer ($\delta$ 5.5 to 6.0) disappeared, confirming that a pure polymer containing halamine and dopamine groups was obtained. The HA and DMA ratio in the polymer chain was calculated by integrating the peaks in the NMR spectrum to be 7:3, which was consistent with the designed feeding ratio of HA and DMA


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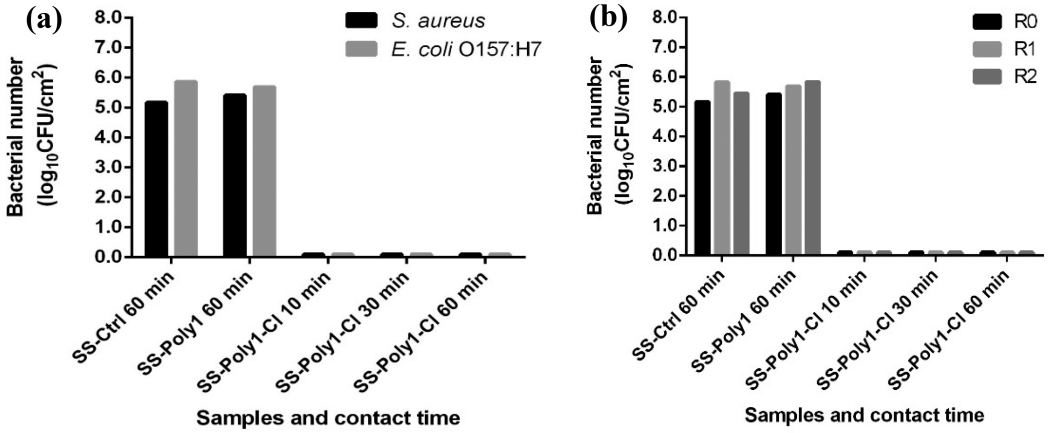


**Figure 3.** Antimicrobial efficacy test on surface: (a) different bacteria, inoculum: *S. aureus* (6.40 $\log_{10}$CFU/sample), *E. coli* O157:H7 (6.32 $\log_{10}$CFU/sample); (b) different recharge cycles, inoculum: *S. aureus* (R0, 6.40 $\log_{10}$CFU/sample; R1, 6.32 $\log_{10}$CFU/sample; R2, 5.88 $\log_{10}$CFU/sample); detection limit: 1.87 $\log_{10}$CFU/sample. SS-Ctrl: uncoated while chlorinated stainless steel; SS-Poly1: halamine−dopamine precursor polymer (poly1, unchlorinated)-coated stainless steel; SS-Poly1-Cl: halamine−dopamine polymer (poly1-Cl, chlorinated)-coated stainless steel.

monomers (7:3). According to previous reports, a DMA content of 20−30% was typically sufficient to provide an adhesive effect;[29] therefore, the obtained p(HA-*co*-DMA) was expected to have sufficient adhesion based on the molecular composition. In addition, GPC results showed that the polymer had molecular weight $M_n$ = 11 957 and $M_w$ = 18 225, with PDI (polydispersity) = 1.52. The FT-IR spectrum (Figure 1b) showed the characteristic peaks of hydantoin (1767 and 1718 $cm^{-1}$) and dopamine groups (1519 $cm^{-1}$). Combined, these data indicated that a halamine−dopamine-functionalized polymer was successfully synthesized. The final polymer product was soluble in a 70−87% EtOH/$H_2O$ solution, which is a convenient solvent for formulating a quick-to-dry, easy-to-apply, and safe coating solution for industrial applications.

316L stainless steel was successfully coated with the halamine−dopamine polymer through dip coating. As shown in Figure 1c, after coating, the surface contact angle of the stainless steel decreased from 72 ± 8° to 59 ± 3°, indicating an increase in surface hydrophilicity. The coating composition was investigated further using XPS (Figure 1d). The appearance of a nitrogen peak (399 eV) on SS-Poly1 (p(HA-*co*-DMA)-coated SS provided evidence that the stainless-steel coupon had been coated with the polymer. Furthermore, the nitrogen peak remained after aggressive chlorination treatment (10% bleach for 30 min), suggesting that the adherence of the coating was relatively strong. XPS also confirmed that activation of the *N*-halamine had been achieved (Figure 2d). Whereas no $Cl_{2p}$ peak (200 eV) was observed on bare stainless steel (SS-Ctrl) and *N*-halamine precursor polymer-coated SS (SS-Poly1), a $Cl_{2p}$ peak (200 eV) was observed after treatment with chlorine bleach. In addition, the $N_{1s}$ peaks shifted and changed shape after chlorination, suggesting that the N−H bond (*N*-halamine precursor) was transformed into a N−Cl bond (*N*-halamine). It should be noted that the N−H groups in dopamine could also be transformed into *N*-halamine (N−Cl), which might contribute to part of the oxidative chlorine content. The coating thickness was estimated via ellipsometry to be between 10 (on silicon wafer) and 50 nm (on stainless steel) depending on the chemical composition of the coating material. SEM micrographs of the surface are also shown in the Supporting Information (Figure S1) and provide additional evidence of successful coating on the stainless-steel surface.

To assess the adhesiveness endowed by the dopamine functional groups, we used an indirect yet simple method based on titration. Because titration determines oxidative chlorines on the surface and chlorine content is positively correlated to the polymer content on the surface, at a certain range, chlorine content on the surface can be used to indicate the quantity of coating polymers attached on the surface. As shown in Figure 2a, for all five different materials tested, PP (polypropylene), HDPE (high-density polyethylene), SS (stainless steel), PVC (polyvinyl chloride), and glass, the chlorine content ($[Cl^+]$) of each material was within the similar range of (2.0 to 5.0) × $10^{15}$ atoms/$cm^2$. Furthermore, the coated and chlorinated stainless-steel coupons (R0) were quenched ("discharged"), rechlorinated ("recharged", R1), and titrated again under the same conditions. Each material adsorbed a similar amount of chlorine on the surface (Figure 2a), indicating that the halamine−dopamine polymers had remained strongly adhered to the surface. The diversity of the material types to which the polymer was found to adhere suggested that the polymer could act as a virtually universal coating regardless of surface chemistry. In addition, the dip-coating methodology was rapid (completed within 5 min for stainless steel) and flexible, able to coat virtually any geometry. Because no special surface treatments were required for this coating method, it is likely to be more easily scalable at a lower cost compared with previously reported methods that were based on layer-by-layer or grafting methods.[22,24]

One of the most attractive properties of *N*-halamine is its "rechargeable" antimicrobial function.[21] As shown in Figure 2b, the chlorine content decreased from 4.0 × $10^{15}$ atoms/$cm^2$ to 3.0 × $10^{15}$ atoms/$cm^2$ after 10 "discharge−recharge" cycles on the SS substrate. This result indicated that whereas a certain amount of coating polymers detached from the coating surface after 10 cycles of aggressive bleach treatment, the remaining layer was strongly attached to the surface. This strong adhesive effect can be explained by the fact that catechol structure within dopamine group can form covalent bonds on solid surfaces, especially metals.[27] This also indicates that the coating is sufficiently robust to withstand periodical treatment of bleach and sanitation washing in real applications.


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Previously, studies on *N*-halamine commonly used oxidative chlorine content for predicting antimicrobial function. Contact killing tests showed significant antimicrobial function when the chlorine content was titratable with the iodometric thiosulfate method (>$10^{15}$ atoms/cm$^2$). As shown in Figure 3a, after 1 h of contact, there were 5.0 to 6.0 $\log_{10}$ CFU/cm$^2$ of *S. aureus* and *E. coli* O157:H7 remaining on the surface of bare stainless steel (SS-Ctrl) as well as the SS coated with the *N*-halamine precursor polymer (SS-Poly1). This is inconsistent with our designed inoculation level of $1 \times 10^6$ CFU/sample (1 in$^2$). However, after activating the *N*-halamine with chlorine, the surface inactivated all inoculated *S. aureus* (Gram-positive) and *E. coli* O157:H7 (Gram-negative) bacteria (>6 $\log_{10}$CFU/cm$^2$) to a level under the detection limit (1.87 $\log_{10}$CFU/sample) within as short as 10 min of contact. To confirm that this potent antimicrobial function was "rechargeable", the surfaces were "recharged" and subjected to two more antimicrobial tests. The N−Cl group is the key for bacterial killing, and the mechanism has been previously reported.[20] In principle, the oxidative power of "$Cl^+$" from the N−Cl will cause damage to the cell wall, similar to the oxidative chlorines in hypochlorite ($ClO^-$). The "$Cl^+$" is expected to be reduced and released from the bond as chloride "$Cl^-$" after killing bacteria. This is the main reason for recharging the coating with hypochlorite ($ClO^-$). Because it has been reported that the antimicrobial function of *N*-halamine is nonspecific (effective against bacteria, fungi, yeast, virus, endospores, etc.),[20,33] only *S. aureus* was used for the following test. As shown in Figure 3b, a similar trend was observed for both SS-Ctrl and SS-Poly1 samples, confirming the consistency of the experimental method. Within the first three "charging" cycles, the *N*-halamine-coated surface was able to inactivate more than 6 $\log_{10}$ CFU/cm$^2$ of inoculated bacteria to a level under detection limit (1.87 $\log_{10}$ CFU/cm$^2$). This was also in good agreement with the titration results that the chlorine content remained stable within the initial three cycles (R0, R1, R2). All of these results combined confirmed that the *N*-halamine-modified surface exhibited potent and nonspecific antimicrobial function, and the antimicrobial function could be "recharged" through treating the surface with chlorine bleach. By contrast, other previously developed materials (e.g., silver alloy/composite compounded polyurethane conveyor belt) achieved only a 2 to 3 log reduction within 24 h of contact.[19]

The "sandwich" test is only effective to measure the ability of surfaces to kill bacteria that are already on the surface. It does not provide any information about the ability of the surface to resist the attachment and proliferation of bacteria from solution. Therefore, additional tests to evaluate the attachment and survival of bacteria on the coated surface from solution were also performed. Combined with the "sandwich" test data, these results are likely to provide a better prediction of performance than the "sandwich" test alone. As shown in Figure 4, after 3 h of incubation in LB medium, many bacteria attached and survived on bare stainless steel (SS-Ctrl) and *N*-halamine precursor-coated stainless steel (SS-Poly1), and bacterial aggregates were observed on the surfaces (green florescent dots with diameter between 1 and 5 $\mu$m represent attached and surviving bacteria). However, for *N*-halamine-activated coating samples, almost no live bacteria could be observed. Because *N*-halamine usually has very fast killing, bacteria may be killed immediately after contacting the surface. Thus there is little chance for bacteria attachment on the surface until the immobilized chorines on the surface are totally consumed.[20,33] This is different from the situation where bacteria colony or biofilm is already formed, and dead cells will still attach to the surface after treatment. Although there could also be some synergistic effect from the polymer coating that can prevent attachment onto the surface, the antimicrobial killing from *N*-halamine plays the major role. This was confirmed with another study of *N*-halamine on stainless steel.[22] The results showed that *N*-halamine exhibits excellent antimicrobial effects for at least 3 h under organic loads (LB medium contains protein and amino acids from peptone and yeast extract). This observation is consistent with previous results that *N*-halamine compounds exhibit excellent biocidal function under heavy organic loads including TSB (tryptic soy broth), meat exudes, and chicken litter.[31−33] However, we acknowledge that the 3 h contact period could only give information of a relative short-term organic load challenge. We did not test longer time (>3 h) in this study because we proposed this coating primarily for nonfood contact surface applications. These surfaces were not likely to be continually challenged with heavy organic load for a long period of time (>3 h) in real applications. In addition, the antimicrobial function may also be partly contributed from the fact that the surface was covered by a polymer coating, which also changed the surface topography and surface tension; all of these factors may also affect the attachment of the bacteria on the surface.

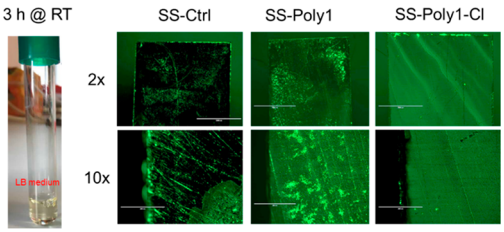


**Figure 4.** Antimicrobial control test of bare stainless steel (SS-Ctrl), halamine−dopamine precursor polymer-coated stainless steel (SS-Poly1), and halamine−dopamine polymer-coated stainless steel (SS-Poly1-Cl) in LB medium. Bright green dots with diameter between 1 and 5 $\mu$m represent attached and surviving bacteria (*S. aureus*).

Because this coating technology was designed to be directly translatable for the application in an industrial environment, its scalability was a key concern. Conveniently, this coating material can be applied to surfaces through spray coating. Compared with previous reports, the chlorine content of our coating is still relatively low. This is because the coating is very thin due to the method of application (dip coating). There are several ways to improve the chlorine content, including increasing the chlorination time or increasing the coating thickness. Dip coating is limited to producing a single layer (10−100 nm) of polymer coating on the surface. To increase the thickness and thus achieve a chlorine content at the level of $10^{16}$ to $10^{17}$ atoms/cm$^2$, we took advantage of the fact that the dopamine group can also be cross-linked (Figure 5a). Catechol moieties in the dopamine structure can react with polyethylenimine (PEI) through Michael addition and Schiff base formation to generate a cross-linked structure (covalent bonds).[31,32] In this study, the successful incorporation of PEI into the dopamine−halamine coating matrix was confirmed through XPS (Figure 5b) and FT-IR (Figure 5c). XPS showed that within the PEI cross-linked polymer matrix, the nitrogen content increased, indicating high nitrogen content compared with HA-co-DMA. The FT-IR spectra showed the character-


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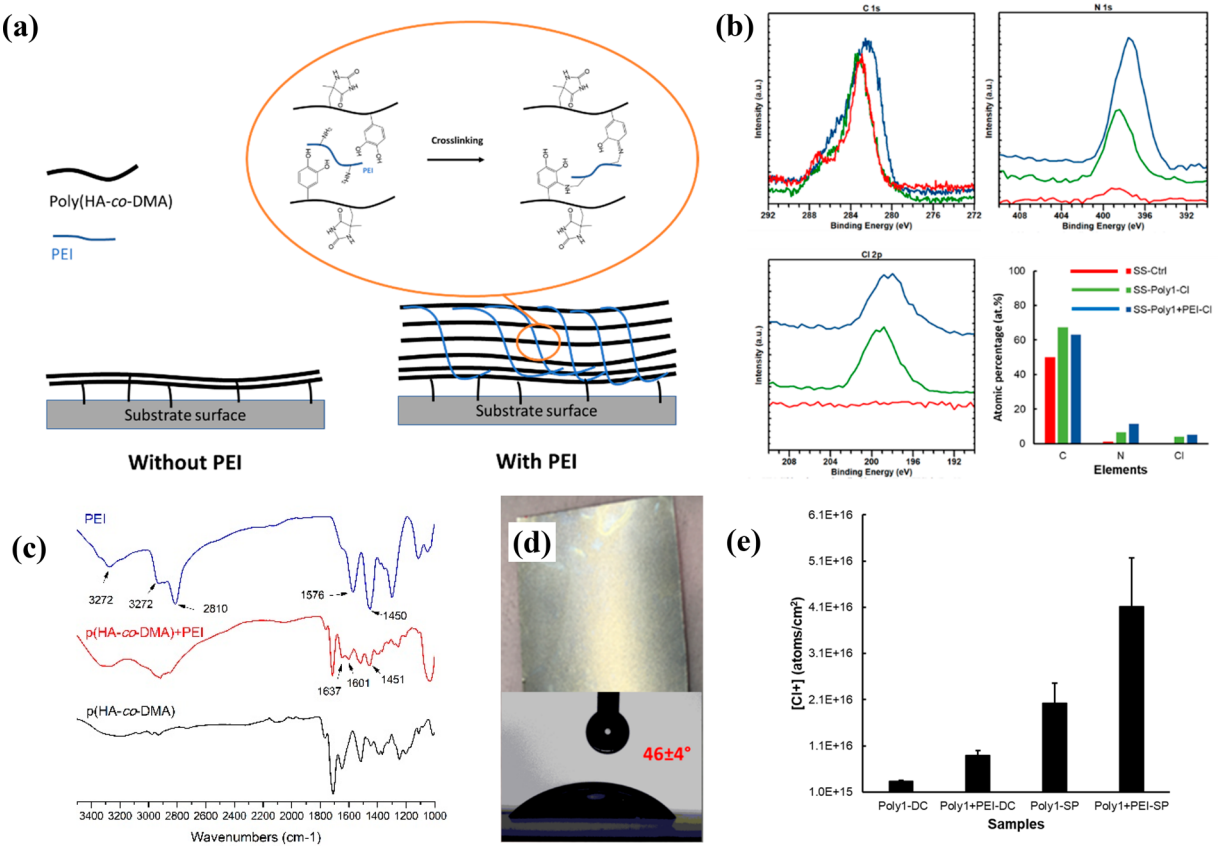


**Figure 5.** Tuning coating thickness and chlorine content through cross-linking: (a) mechanism of cross-linking with PEI; (b) XPS analysis of C, N, and Cl and their atomic percentage (at %) on bare stainless steel (SS-Ctrl), un-cross-linked (SS-Poly1-Cl), and PEI cross-linked coating (SS-Poly1+PEI-Cl); (c) FT-IR spectra of PEI, p(HA-co-DMA), and PEI cross-linked p(HA-*co*-DMA)+PEI coating; (d) appearance and contact angle of PEI cross-linked coating (SS-Poly1+PEI) through dip-coating method; and (e) oxidative chlorine content of p(HA-*co*-DMA) (poly1) and poly1+PEI-coated surfaces through dip-coating (DC) and spray-coating (SP) methods.

istic peaks of PEI at 3272 and 1450 $cm^{-1}$ in the cross-linked coating. Also, the peaks at 1601 and 1637 $cm^{-1}$ identified the presence of —C═C— and —C═N— bonds, respectively, additional evidence of cross-linking. Interestingly, it was observed that after adding PEI into the formulation, the coating changed from colorless to reddish brown (Figure 5c), and the contact angle further decreased to ~46 ± 4°, probably due to the increase in the number of N−H and $-NH_2$ groups. Titration results confirmed that the cross-linked coating had a higher capacity for binding chlorine. This could be explained by the fact that more *N*-halamine precursor polymer (HA-co-DMA) molecules were attached onto the surface, which resulted in thicker coating. In the case of dip coating, the chlorine content of the PEI cross-linked coating increased almost more than two-fold compared with the pristine coating, reaching $10^{16}$ atoms/$cm^2$. In the case of spray coating, it reached an even higher value. Spray coating without PEI had chlorine content of $2 \times 10^{16}$ atoms/$cm^2$, whereas the PEI cross-linked coating had chlorine content of $4 \times 10^{16}$ atoms/$cm^2$. The chlorine content could be further increased by tuning several other parameters including polymer concentration, spray-coating times, chlorination conditions, the number of coating layers, and so on.

We applied the coating onto a metal pipe as a sample piece of real food-processing equipment. To simulate realistic conditions, the metal pipe was taken directly from a food-processing plant, and the surface was scrubbed to damage it, as such defects have a higher tendency to harbor microorganisms (Figure 6). As shown in Figure 6b, the coating was applied via the spray coating method, resulting in a darker color. Although in initial experiments a 10% bleach solution was used to activate the *N*-halamine, a lower concentration is more commonly used to sanitize surfaces in food-processing plants. Therefore 0.5% bleach (about 200−300 ppm of free chlorine) was sprayed to chlorinate the surface. The coated layer was more hydrophilic than the bare metal pipe, allowing the bleach to uniformly wet the entire surface. Because a low concentration of bleach (200−300 ppm available chlorines) was used and it took only 10 min until the coated part was fully dried, there was likely to be only a minimal effect of corrosion. To reduce any possible corrosion even further, the surface could be rinsed with potable water following sanitization. We also estimated that based on the current coating method the halamine−dopamine polymer on the surface was 130 mg/$m^2$ and the final cost of manufacturing and applying this halamine−dopamine polymer would be about $2 USD/g. Thus the final cost of applying this polymer coating would be around $0.26 USD/$m^2$. which may be considered a relatively low cost for antimicrobial coatings.

In an industry environment it is important to ensure that the effectiveness of the coating does not diminish over time. Therefore, we designed a facile and rapid method for validating that the chlorine "recharging" process occurred correctly based on titration. An indicator solution was designed to turn blue within 5 s when spread on the surface if chlorine was present (Figure 6d). For convenience, the indicator solution can be


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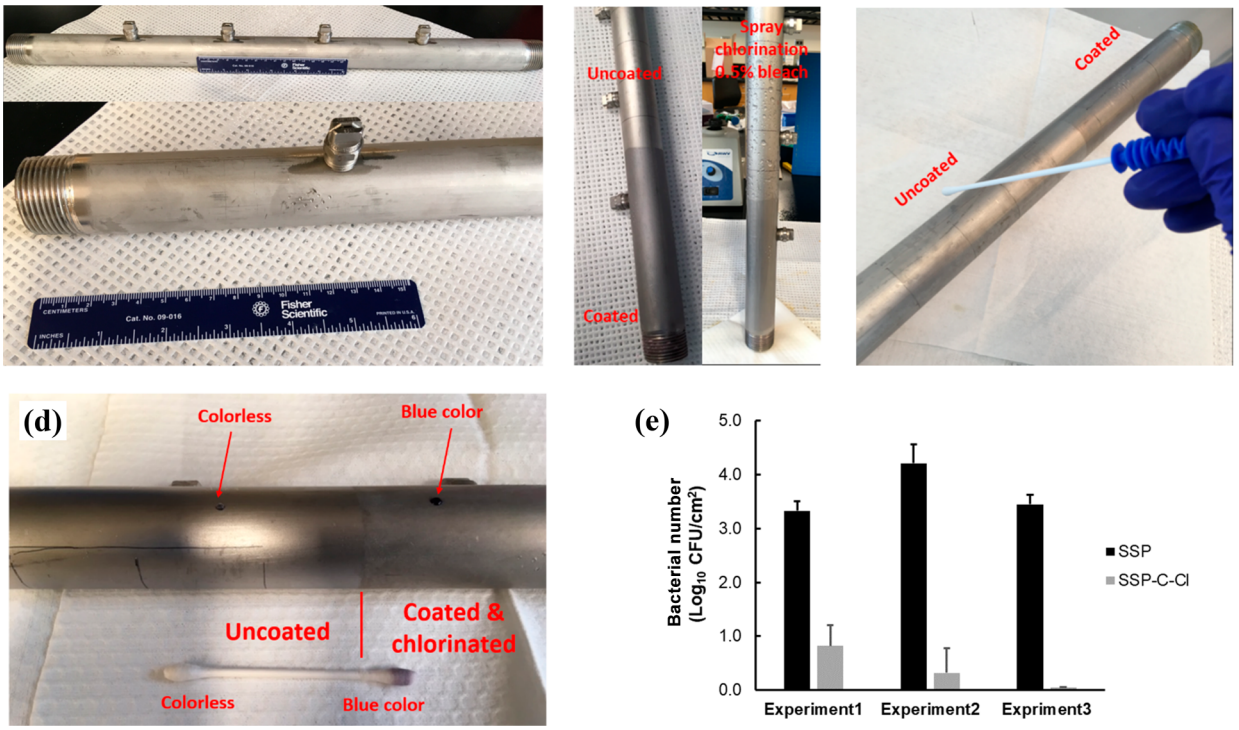


**Figure 6.** (a) Appearance of a used SS 304 pipe. (b) Appearance of spray-coated SS pipe after drying and during spray chlorination with 0.5% bleach. (c) Inoculating bacteria on coated SS pipes. (d) Color-based indication of immobilized oxidative chlorines on SS surface. (e) Antimicrobial control effect of *N*-halamine coating on SS pipe surface, inoculum bacteria: *S. aureus*. Experiment 1, 8.83 $\log_{10}$CFU/mL; Experiment 2, 8.07 $\log_{10}$CFU/mL; Experiment 3, 8.14 $\log_{10}$CFU/mL. For all three experiments, sample size $N = 3$. SSP: stainless steel pipe; SS-C-Cl: stainless steel coated with halamine−dopamine polymer and chlorinated with 0.5% bleach.

applied to a cotton swab. Upon swabbing the surface, the cotton would turn blue. In the future, a colorimetric indicator scale could provide semiquantitative information on the chlorine concentration.

We confirmed the antimicrobial effect of the coating on a real equipment part, inoculating the surface with bacteria and measuring the number of living microbes in three separate sequential experiments (Figure 6c). As shown in Figure 6e, on the uncoated part of the SS pipe (SSP), about 3.0 to 4.0 $\log_{10}$CFU/cm$^2$ bacteria (*S. aureus*) could be recovered from the surface after 1 h of contact. However, for *N*-halamine-coated surfaces, <1.0 $\log_{10}$CFU/cm$^2$ could be recovered. The antimicrobial function was improved with additional chlorination in experiment 2 and experiment 3. In experiment 3, no culturable bacteria were recovered from the *N*-halamine-coated surface. Because in experiment 1 the coating was not sufficiently charged with 0.5% bleach, more charging cycles resulted in higher chlorine loading and better antimicrobial function. This result also confirms that the coating can sustain several cycles of sanitizing and washing without losing antimicrobial function considering the fact that the coating on the pipe surface had been treated with chlorine bleach at least 9 times and thoroughly washed about 15 times with DI water during the experimental procedure.

Although *N*-halamine has been shown to perform reasonably well under conditions of high organic load, it is possible that in applications in which the coating had direct contact with food items the antimicrobial efficacy could be reduced compared with what has been reported here. We intend to perform additional studies in the future to test the antimicrobial efficacy of the coating in the presence of actual food items. However, in some areas of food manufacturing (such as zone 1 food equipment), non-food-contact food equipment surfaces are not heavily soiled or fouled by organic load; therefore, this is likely not a concern for application in these areas. In accordance with the general food plant sanitation philosophy that any improvement strategies for increasing food safety should supplement rather than replace current strategies, the coating would be implemented in addition to current sanitation methods.

The immediate next step is to test the coating in a pilot-scale food-processing plant. Thus the safety of the coating itself, not merely its efficacy, is a concern. It has been widely accepted that *N*-halamine and dopamine are safe structures.[16,26] Historically, the biggest hindrance that prevents the application of coatings on direct food-contact surfaces is that the coatings must be so thick to achieve a desired function that the coating will tend to flake off with time, becoming a food adulterant and causing another food safety issue. In the case of our coating, a thickness of only several hundred nanometers was needed to achieve the desired function. Our data suggest that such a coating is relatively stable and unlikely to peel off the surface. However, more studies to challenge the coating under commercial food plant sanitations, such as hot and high-pressure water washing, strong acid or alkline agents, degrease agents, and sanitizers (QACs), should be studied. In this research, we mainly focused on nonfood contact environmental surfaces. Although this coating can also be used for food-contact surfaces, further toxicity studies and recognition from regulatory agencies (e.g., U.S. FDA Food Contact Notification Program) are warranted. For food-contact surface applications, leaching of toxic products from coatings into food products needs to be considered. The only possible leaching product is chlorine or chloride; however, the amount is really


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small, even under wet conditions.[20] In addition, it is known that chlorine bleach has been approved for food-contact surface sanitizing applications; these have been known to be safe for food contact.

In conclusion, in this research, we developed a polymer designed with food industry applications in mind. This polymer coating can be applied to both food contact (working table, cutting knife, container, conveyor belts, etc.) and environmental surfaces (nonfood contact equipment parts, walls, ceilings, drainages). We showed that the coating adheres to a variety of substrates regardless of chemical composition, size, or shape. Depending on the application, different coating methods (dip-coating and spray-coating) could be chosen, and the total coating procedure was achieved within 5−10 min for either method. Furthermore, the chlorine "charging" process could be incorporated into current sanitization procedures, thus very little change in food-processing protocols would be needed to implement the coating system. We also demonstrated the efficacy of the coating on real food equipment parts and also developed a simple and low-cost procedure for validating the success of the coating and chlorination processes. The versatile and rechargeable coating material developed in this study has great potential for food safety preventive-control applications in the food industry.

## ■ ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jafc.8b03864.

Synthesis of halamine vinyl monomer HA. Synthesis of dopamine vinyl monomer (DMA). Figure S1. SEM of stainless steel and coating. (PDF)

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### Notes

The authors declare no competing financial interest.

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