



N-halamine surface coating for mitigation of biofilm and microbial contamination in water systems for space travel

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ABSTRACT

A copolymer termed HASL produced from monomeric units of 2-acrylamido-2-methyl-1-(5-methylhydantoinyl) propane (HA) and of 3-(trimethoxysilyl)propyl methacrylate (SL) has been coated onto stainless steel and Inconel™ substrates, which upon halogenation with either aqueous oxidative chlorine or bromine, became antimicrobial. It has been demonstrated that the halogenated stainless steel and Inconel™ substrates were effective in producing 6 to 7 log inactivations of *Staphylococcus aureus* and *Escherichia coli* O157:H7 within about 10 min, and in prevention of *Pseudomonas aeruginosa* biofilm formation over a period of at least 72 h on the stainless steel substrates. Upon loss of halogen, the HASL coating could be re-charged with aqueous halogen. The HASL coating was easily applied to the substrates via a simple dip-coating method and was reasonably stable to contact with water. Both chlorinated substrates could be loaded with at least 6×10^{16} oxidative Cl atoms per cm^2 and maintained a loading of greater than 1×10^{16} chlorine atoms per cm^2 for a period of 3–7 days while agitated in aqueous solution. After loss of chlorine to a level below 1×10^{16} atoms per cm^2 , the substrates could be recharged to the 6×10^{16} Cl atoms per cm^2 level for at least 5 times over a 28 day period. The new antimicrobial coating technology has potential for use in a variety of important applications, particularly for water treatment and storage on spacecraft.

1. Introduction

Water is one of the consumable supplies required by humans who live, work, and travel in outer space. At least initially, all of the water required must be transported with the humans as they travel from earth to outer space. The minimum amount of water per day to support human life is about 3.5 L per person, and adding a bit for cleaning *etc.*, brings the total to about 4 L or 1 gal per day per person. Transportation costs and storage space requirements for this amount of water dictate that most of the water consumed must be recycled rather than discarded into outer space. Recycling of water from human waste into a potable stream and storing the water for an extended period of time, such as for the International Space Station Water Recovery System, present problems. In particular, water storage in tanks and distribution lines allows for the development of microbial growth, which over time produces biofilm, often described as a slime, which blocks pipes and valves and makes the

water unpalatable and possibly unsafe. Biofilm formation is a multistage process, starting with the attachment of bacteria aggregates onto a substrate surface. Once the complex multicellular biofilm structure is attached and established, it is difficult to destroy the biofilm bacterial populations [1–3]. In fact, most antimicrobial compounds and antibiotics are not able to effectively eliminate biofilm populations [4–6]. Since the use of large amounts of gaseous antimicrobials, such as chlorine and ozone, are impractical and unsafe on space stations and spacecraft, it would seem that the best approach for eliminating pathogens and biofilms in space would be to employ effective antimicrobial coatings on the water-treatment and water-storage equipment. Unfortunately such coatings developed to date, such as those polymers containing metals like silver or quaternary ammonium compounds, are not effective for the intended application in space because of low efficacies against the bacteria and biofilms encountered and replacement after the antimicrobial activities are exhausted.

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The current study regards mitigation of biofilm formation and build-up in water systems during storage or usage in space travel. The main goal of this work was to develop an antimicrobial N-halamine coating that could be chemically bonded onto the surfaces of the metal substrates (stainless steel and Inconel™) used in spacecraft water systems, and which, would inhibit the growth of microorganisms and prevent biofilm formation.

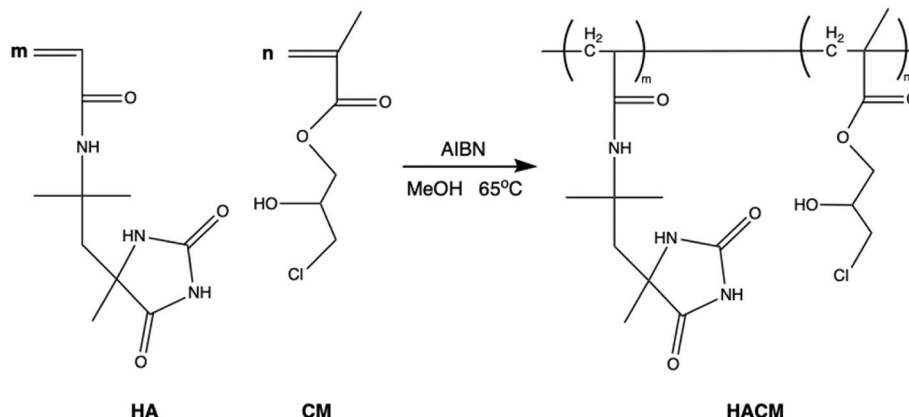
Since the early 1980's N-halamine antimicrobial materials and polymers have been developed and studied extensively in these laboratories [7,8] and increasingly elsewhere [9,10]. These N-halamine compounds generally contain oxidative chlorine or bromine atoms covalently bonded to nitrogen atoms in the molecules. Since the halogen atom retains a +1 oxidation state, it is capable of inactivating pathogenic microorganisms. Those N-halamines which have been developed in these laboratories have been designed to contain sufficiently strong N-Cl or N-Br chemical bonds so as to dissociate minimally when the compounds are exposed to aqueous solution. Their mechanism of action against pathogens is a direct transfer of the oxidative halogen atom to the cell upon contact, followed by an oxidation process of critical components of the cell, such as sulfhydryl groups, leading to inactivation of the pathogens. The N-halamine compounds have been shown to be effective at inactivation of bacteria, fungi, protozoa, and virus particles [7,8]. N-halamines generally possess contact inactivation times which are much lower than those for other antimicrobial compounds such as quaternary ammonium salts and metals such as silver. Also, the N-halamines can be regenerated upon loss of their oxidative halogen due to pathogenic inactivation, reaction with reducing agents, or organic materials, by simple exposure to a source of aqueous free halogen.

N-halamine polymer coatings have been grafted onto a variety of surfaces in order to achieve antimicrobial activity. Such surfaces include commercial polymers such as poly(styrene) to be used in antimicrobial water filters [11–14], various types of fibers, in particular cotton [15, 16], poly-ester [16,17], and poly(propylene) [18,19], and various materials used in food preparation surfaces [20]. However, coating of metals such as stainless steel with an N-halamine polymer presents a formidable challenge due to the necessity of bonding sites on the metal surface and appropriate linking groups. Nevertheless, there have been several prior reports concerning N-halamine coatings on stainless steel, although none, to our knowledge, concerning Inconel™.

The first reports of attachment of an N-chloramine moiety on the surface of stainless steel were from the laboratories of Bastarrachea and Goddard [21]. In that work multilayers of branched poly(ethyleneimine) and poly(acrylic acid) were attached to the surface through a silane coupling agent after the surface had been treated with piranha solution (a mixture of hydrogen peroxide and sulfuric acid) to create surface hydroxyl groups. The chloramine was then produced *in situ* by exposure to dilute sodium hypochlorite. This treatment provided a 1.5 log reduction of *Listeria monocytogenes* at a contact time of 6 h. Earlier

work in these laboratories utilized a copolymer (HACM in Scheme 1) of 2-acrylamido-2-methyl-1-(5-methylhydantoinyl)propane (HA) and 3-chloro-2-hydroxypropyl methacrylate (CM) to bond to the surface of stainless steel [22]. The monomer HA was chosen because it contains three nitrogen sites for bonding to

oxidative halogen, an imide and amide site in the hydantoin ring and an acyclic amide site not subject to a dehydrohalogenation reaction because of the two methyl groups on carbon 2 of the propane backbone. The stainless steel surface in this case was also treated with piranha solution to create surface hydroxyls and then with aqueous NaOH to facilitate the S_N2 reaction between surface O^- and the terminal CH_2 group on CM. After exposure to dilute sodium hypochlorite, this treated stainless steel surface was able to load between 5.0×10^{16} and 1.5×10^{17} oxidative chlorine atoms/cm². It provided 6 log reductions of *Staphylococcus aureus* and *Escherichia coli* O157:H7 within 15 min of contact and was able to survive at least five cycles of rinsing with water under identical conditions to the methodology employed in the research to be presented herein, which will be described in the Materials and Methods Section. Ma and coworkers have also employed the same monomer HA with the monomer dopamine methacrylamide to create a copolymer having four nitrogen sites which they bonded to several types of surfaces including stainless steel [20]. The dopamine methacrylamide monomer was synthesized by a literature method. Its fourth nitrogen binding site upon chlorination could be subject to a dehydrohalogenation reaction; therefore, the copolymer effectively has three nitrogen binding sites. Their chlorinated surface loaded 2.0 to 5.0×10^{15} Cl atoms/cm² and provided 6 log reductions of the bacteria mentioned above within about 10 min. Their coating was reasonably stable to up to ten discharge-recharge cycles, and they believe that it would be effective in food preparation applications. Liu and coworkers have employed an interesting version of “click chemistry” to attach a quaternized azido hydantoin to stainless steel [23]. Upon chlorination, a mixed quat/N-chloramine coating was obtained. A pretreatment with piranha solution, and then a silanizing agent, was required. The surface achieved a 5.3 log reduction within a contact time of 1–2 h. Rai and coworkers have also bonded a copolymer containing three nitrogen sites to stainless steel [24]. Their copolymer involves the monomers (Z)-N-((4-(2,5-dioximidazolidin-4-ylidene)methyl)phenyl)methacrylamide containing a hydantoin ring and 3-(methacryloyloxy)propyltrimethoxysilane as the surface linking agent. The treated surface provided 6 log reductions of *S. aureus* and *E. coli* in contact times of 10–12 min. It also showed anti-biofilm activity. However, a pretreatment with piranha solution was performed, and the synthesis of the hydantoin monomer, which was present in the copolymer at 10 mol percent was tedious, and probably not practical. Huang and coworkers also evaluated the efficacy of a water-soluble monomeric N-chloramine 1-chloro-2,2,5,5-tetramethyl-4-imidazolidinone deposited on the surfaces of several materials including stainless steel employed in broiler chicken houses [25]. They



Scheme 1. The Reaction of HA and CM to Form the Copolymer HACM for Coating on Stainless Steel.

found that 1 wt percent of the compound on the surfaces could inactivate 6 logs of *Salmonella typhimurium* and *Campylobacter jejuni* within 2 h contact and that the substance was still active at 28 d even though the oxidative chlorine content had decayed from about 4×10^{16} to about 5×10^{15} atoms/cm². In this case the monomer was not chemically bound to the surfaces and could therefore be removed by exposure to water.

In the current work the monomer HA discussed above was copolymerized with 3-(trimethoxysilyl)propyl methacrylate (SL) as shown in Scheme 2. The SL linking agent in this copolymer, henceforth designated as HASL, will be shown to bond strongly to both stainless steel and Inconel™ without extensive surface pretreatment. Data will be presented concerning antimicrobial efficacies created after both chlorination and bromination. Wet stability and regenerability studies and efficacies against biofilms will also be reported. The latter are particularly relevant to the intended application for coatings of water-contact equipment in spacecraft.

2. Materials and methods

2.1. Materials and instrumentation

2-Acrylamido-2-methyl-4-pentanone was purchased from Tokyo Chemical Industry, Co., LTD (Tokyo, JP). 3-(trimethoxysilyl)propyl methacrylate, potassium cyanide, and ammonium carbonate were obtained from Acros Organics (NJ, USA). 2,2'-Azobis(2-methylpropionitrile) (AIBN) was obtained from Sigma-Aldrich, Inc. (St. Louis, MO, USA). All other chemicals were purchased from Aldrich Chemical Co. (Milwaukee, WI), TCI America (Boston, MA), or Alfa Aesar (Ward Hill, MA) and used as received unless otherwise noted. Highly corrosion resistant stainless steel 316L having thickness 0.008 inch (ASTM A240/A240 M standard) with 2B finish (Ra roughness of the surface typically being between 0.3 and 0.4 μm) and Inconel™ 718 alloy sheet with 0.02 inch thickness were purchased from McMaster-Carr (Chicago, IL). ¹H NMR spectra were obtained with a Bruker 400 MHz spectrometer (16 scans). ATR-IR data were recorded with 32 scans at 4 cm⁻¹ resolution with a PerkinElmer Model Spectrum 400 ATR-FTIR spectrometer. Melting points were determined by differential scanning calorimetry (DSC, TA Instruments Model Q-2000). Mass spectra were obtained with a Waters Q-TOF Premier spectrometer, and X-ray photoelectron spectra (XPS) were obtained with a Kratos XSAM 800 Surface Analysis System equipped with a hemispherical energy analyzer.

2.2. Methods

2.2.1. Synthesis of HA

The monomer 2-acrylamido-2-methyl-1-(5-methylhydantoinyl)propane (HA) was synthesized according to a Bucherer-Berg protocol as described earlier [15,26]. Briefly, HA was synthesized by reacting

2-acrylamido-2-methyl-4-pentanone, potassium cyanide, and ammonium carbonate in a 1:2:6 M ratio in a water/ethanol (1:1 v/v) solvent mixture in a round-bottom flask at room temperature for 5 d. After evaporation of ethanol, the crude product was isolated by adjusting the pH to 7 using dilute 6 N HCl and collecting a white powder after filtration. The HA monomer was recrystallized from acetonitrile and dried at 45 °C for 24 h prior to the HASL copolymer reaction.

2.2.2. Synthesis of HASL

The HASL copolymer was synthesized by free radical polymerization as shown in Scheme 2 above. In a 100 mL round-bottom flask, equimolar amounts of 3-(trimethoxysilyl)propyl methacrylate (SL) (10 mmol) and HA (10 mmol) were dissolved in methanol. After 1 wt % azobis(isobutyronitrile), AIBN, was added to the flask, nitrogen gas was bubbled through the solution for 15 min to remove any dissolved oxygen before initiating the reaction. The mixture was refluxed at 65 °C for 2 h in the presence of the AIBN initiator under a nitrogen atmosphere. After completion of the copolymer reaction, the solvent was evaporated, and the HASL copolymer was obtained as a white solid.

2.2.3. Composition of stainless Steel and Inconel™ substrates

The compositions of corrosion resistant stainless steel 316L and Inconel™ 718 alloy sheet, henceforth referred to as SS and IN, respectively, used in this study are shown in Table 1. The data were supplied by McMaster-Carr, Inc. (Chicago, IL).

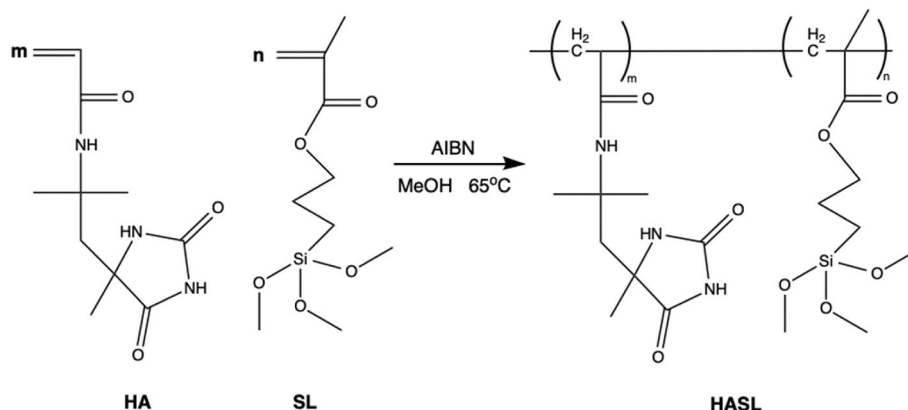
2.2.4. Pretreatment of the metal surfaces

The SS and IN sheets were cut into 2.54 × 2.54 cm coupons. To compare and test the adhesion capability of the coatings on SS and IN metal substrate surfaces, two types of cleaning procedures were applied prior to surface modification with the synthesized HASL copolymer. All of the coupons were subjected to solvent cleaning and in some cases an oxidation process utilizing acid etching. The cleaning process involved immersion into first acetone, then ethanol, and finally deionized water, in each case while agitating at 150 rpm for 15 min at 25 °C, to remove oil and dirt. The cleaned coupons were then dried under nitrogen flow to avoid stains on their surfaces followed by drying at 45 °C for 1 h. They were then placed into sealed poly(propylene) bags and held under vacuum until the coating procedure was initiated. To compare the HASL adhesion characteristics of the SS coupons with and without acid etching to promote oxidation of the surface, a portion of the SS coupons were

Table 1

Compositions of the 316L SS and 718 in used in this study.

Substrate	% C	% Fe	% Si	% Cu	% Ni	% Cr	% Other
316L SS	<0.08	58–74	<1	<1	10–15	18.5	<7
718 IN	0.03	17.56	0.06	0.1	53.62	18.6	<10



Scheme 2. The Reaction of HA and SL to Form the Copolymer HASL for Coating on Stainless Steel and Inconel™.

exposed to piranha solution (30% H₂O₂/H₂SO₄ in 7:3 volumetric ratio) under agitation at 150 rpm at 25 °C for 30 min. They were then rinsed with copious amounts of deionized water, then dried and stored in the same manner as for the non-acid SS coupons. The IN coupons were not exposed to acid etching.

2.2.5. Coating of the metal surfaces with HASL

A dip-coating method was used for modification of the metal surfaces of the coupons. In this procedure the 6.45 cm²

SS and IN coupons were immersed in 3 wt % HASL copolymer solution prepared in ethanol/water at a 3:1 volumetric ratio. The coupons were agitated at 170 rpm for 30 min at 25 °C. Following treatment with the N-halamine precursor HASL copolymer, each coupon was dried under nitrogen atmosphere and then cured using a heat gun for 1 min followed by oven heating at 130 °C for 30 min to promote covalent bond formation between the substrate surface and the HASL copolymer.

2.2.6. Activation of the coated coupons with oxidative halogen

After the coating process, HASL copolymer-functionalized coupons were then subjected to a halogenation process in order to convert the N-H sites to the N-Cl or N-Br moieties that provide antimicrobial properties. Schematic representation of the HASL coating formation onto stainless steel and the chemical structure of the N-halamine moieties following the halogenation process is shown in Scheme 3. For chlorination, an aqueous solution of household bleach (8.75% sodium hypochlorite) was prepared by 10-fold dilution in deionized water. The HASL-coated SS or IN coupons were chlorinated in this 10% (v/v) aqueous solution of household bleach at a pH of 7 (adjusted by 4 N HCl solution) for 30 min at 25 °C. After the chlorination process, the coupons were rinsed in deionized water and finally dried at 45 °C for 1 h to remove any occluded free chlorine from the material. After the chlorination reaction, the coupons were labeled as SS-HASL-Cl and IN-HASL-Cl. For bromination, a 2 N NaOH aqueous solution was prepared in deionized water, and a measured amount of liquid bromine was added into the mixture. The pH of this solution was adjusted to 6.04 with 4 N acetic acid solution. Then HASL coated coupons were immersed in this solution for 30 min at 25 °C. Following the N-bromination reaction, the brominated coupons were rinsed in deionized water and then dried at 45 °C for 1 h. These coupons were labeled as SS-HASL-Br and IN-HASL-Br.

2.2.7. Analytical determination of halogen loading on the coated coupons

The oxidative halogen contents of the N-halamine-modified coupons were determined by standard iodometric/thiosulfate titration. Briefly, the N-halogenated HASL coated coupons were placed into a flask containing an aqueous solution prepared with excess KI added. The flasks were then sealed with a stopper and agitated with a magnetic stirrer for 15 min at 25 °C. Then 2 N acetic acid and 0.5 w/w % soluble starch were

added into the mixture to produce a dark blue color, and the clear endpoint was determined by titration with 0.00375 N sodium thiosulfate solution. The available chlorine and bromine atoms/cm² were calculated using the following equation:

$$\text{Total halogen content } \left(\frac{\text{atoms}}{\text{cm}^2} \right) = \frac{N \times V}{2A} \times 6.02 \times 10^{23} \quad (1)$$

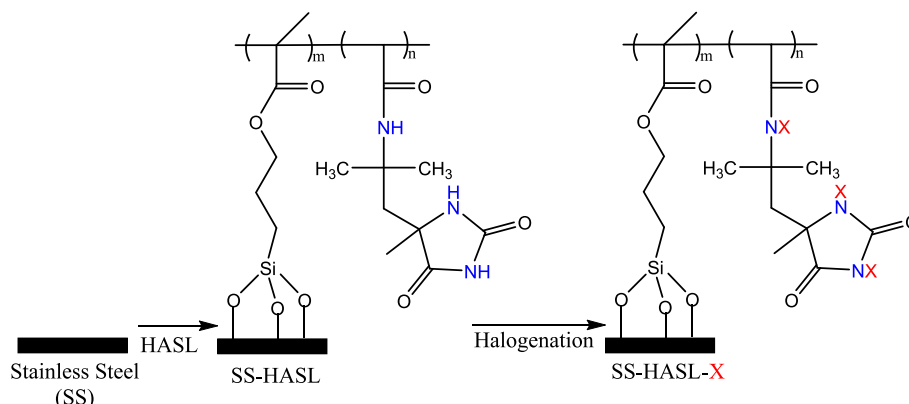
where N and V are the normality (equiv/L) and volume (L) of the titrant (Na₂S₂O₃), respectively, and A is the surface area of both of the sides of the SS or IN coupons. The 2 in the denominator refers to the two electron change from oxidative halogen atoms during the redox titration. Neither the surface area of the thin coupon edges nor the surface roughness were considered as contributing to the surface area of the coupons, but the error introduced by these assumptions in the calculation of halogen atoms/cm² is judged to be less than 5%.

2.2.8. Wet stability and chlorine regeneration testing

To test adhesion and bonding stability of the HASL coating on the substrate surface, wet stability testing in an aqueous medium was employed. Prior to this test, untreated (no acid etching) cleaned coupons were coated with HASL by the dip coating, drying, and curing method previously described. Then the HASL coated coupons were chlorinated for 30 min. Liqui-Nox™ detergent solution (1 v/v %) was prepared in deionized water, and 50 mL tubes were filled with 40 mL of this detergent solution. Chlorinated HASL-coated coupons (SS-HASL-Cl and IN-HASL-Cl) were immersed in this solution while mixing vigorously with a magnetic stirrer at 25 °C and retained for predetermined time periods of 0, 3, 7, 14, and 28 d. Coupons were removed at the end of each time period, rinsed in DI water, and dried at 45 °C for 1 h. The chlorine remaining on each coupon was measured using iodometric/thiosulfate titration (eq (1)). Once the chlorine was titrated, the coupons were rinsed thoroughly and then subjected to a re-halogenation process in order to determine the HASL coating stability. The chlorine content after re-chlorination was again determined by iodometric/thiosulfate titration. Losses of significant amounts of titrated chlorine following attempted regeneration would be indicative of dissociation of the HASL from the surfaces of the coupons.

2.2.9. Antibacterial efficacy testing

Efficacies of un-halogenated SS, IN, and HASL-modified SS and IN coupons as controls, and the N-halogenated HASL-modified SS and IN coupons were evaluated against Gram-positive *S. aureus* (ATCC 6538) and Gram-negative *E. coli* O157:H7 (ATCC 43895) bacteria by a “sandwich” contact-killing method which has been employed in these laboratories for a variety of material surfaces and has been described previously [16]. In this procedure the bacteria were suspended in 100 μM phosphate buffer (pH 7) to produce a suspension of known population (colony forming units, CFU). Each strain of bacteria was streaked



Scheme 3. Coating of HASL Copolymer on SS Followed by Subsequent Halogenation; X is Cl or Br, and an Analogous Process Was Used for IN.

onto a trypticase soy agar (TSA) plate and incubated at 37 °C for 16 h. Bacterial suspensions were prepared by suspending bacterial colonies swabbed from the TSA plates in 100 µM Butterfield's phosphate buffer (BPB). Bacterial populations of the bacterial suspensions were estimated by using a microplate spectrophotometer (Thermo Fisher Scientific, Pittsburgh, PA) at O.D. 640 nm, and inocula with determined populations of about 4×10^7 CFU/mL were prepared. Then, an aliquot of 25 µL of this suspension (containing ca. 6 logs of CFU) was placed in the center of a 2.54 cm × 2.54 cm square coupon (SS, IN, HASL-modified and un-halogenated as controls, or HASL-modified and halogenated as potential antimicrobial samples), and a second identical coupon was placed on top of it. After predetermined contact times (5, 10, 15, and 30 min), the coupons were quenched by 5.0 mL of sterile 0.02 N sodium thiosulfate solution to neutralize any remaining oxidative chlorine and thus terminate the disinfection action. The quenched samples were first vortexed for 2 min, and then 10-fold serial dilutions were prepared using pH 7, 100 µM phosphate buffer. Triplicate dilutions were plated on TSA. After the plates were incubated at 37 °C for 24 h, bacterial colonies were counted for the biocidal efficacy analysis. The log of the number of

various substrate coupons were prepared in quadruplicate and placed into six-well plates. Three mL of bacterial suspension were added on top of each coupon to ensure coverage of the surface of the coupon. The inoculated coupons were then statically incubated at 37 °C for 24, 48, and 72 h to allow bacterial adhesion onto the coupons. After each cultivation contact time, the coupons were removed from their wells using sterile tweezers and gently washed in PBS buffer solution twice to remove loosely adhered bacteria from the surface. Then each coupon was placed into a 50 mL sterile tube, and 20 mL of PBS solution were added. The adherent bacteria attached to the surface of each coupon were collected by ultrasonication for 5 min. The coupons were then vortexed for an additional 1 min at maximum power. An aliquot of 100 µL of this suspension was removed, and 10-fold serial dilutions were prepared using pH 7, 100 µM phosphate buffer. The dilutions were plated on TSA, and the plates were incubated at 37 °C for 24 h. Bacterial colonies were counted for enumerating the number of firmly attached bacteria on the surface of each coupon. The number of viable bacteria (CFU/sample) at each contact time were calculated using eq. (3) below.

$$\log_{10} \frac{CFU}{sample} = \log_{10} \frac{(\text{bacteria colonies counted on agar plate} \times \text{dilution factor} \times 20 \text{ mL})}{\text{plated volume of } 0.075 \text{ mL}} \quad (3)$$

bacteria (CFU/sample) of inoculum and number of viable bacteria at each contact time were calculated as shown below:

The detection limit in this case was 200 CFU per sample.

$$\log_{10} \frac{CFU}{sample} = \log_{10} \frac{(\text{bacteria colonies counted on agar plate} \times \text{dilution factor} \times 5 \text{ mL})}{\text{plated volume of } 0.075 \text{ mL}} \quad (2)$$

Our technique allows the detection of 1 CFU in a 100 µL sample; thus since 100 µL aliquots were withdrawn from the 5 mL quenched solution for plating, the detection limit was 50 CFU per sample. Two replicate coupons were used in each experiment for each type of treatment against one type of bacteria at each determined contact time (5, 10, 15, and 30 min). As a result, in each experiment 24 coupons were tested against one type of bacterium. All experiments were performed at least two times on different days using fresh bacterial inocula for each type of bacterium. Nine coupons were used to measure the amount of oxidative halogen by iodometric titration on the surface of the coupons used in each experiment.

2.2.10. Biofilm formation assay

Bacterial attachment, biofilm formation, and growth by adhesion on the SS substrates were assessed in a manner similar to that from previous work [5,27]. *Pseudomonas aeruginosa* (ATCC 27853) obtained from the American Type Culture Collection (Rockefeller, MD) was used in this biofilm formation assay. SS, SS-HASL, and SS-HASL-Cl coupons were cut to 2.4 × 2.4 cm in size. The SS and SS-HASL coupons were used as controls. All coupons were cleaned in solvents, but not acid etched, as described earlier. Only the three types of SS substrates were studied. Biofilm formation and adhesion onto the SS substrate surfaces were evaluated by using flat bottom sterile six-well cell culture plates. In this procedure the bacteria were suspended in 100 µM phosphate buffer (pH 7) to produce a suspension of known population (colony forming units, CFU). The same method as described in the previous section was used to prepare inocula containing populations of about 3.60×10^6 CFU. The

2.2.11. Biofilm formation and inoculum depletion assay

The procedure used above was followed with the modification that at each contact time (24, 48, and 72 h), the solution that contained the inoculum (in this case 4 mL) was removed, and a new fresh solution of 4 mL of inoculum was added. The same procedure for analyses of bacterial adhesion to the coupons as described above was employed, but in addition, after each contact time before addition of new inocula, 100 µL aliquots of bacterial suspension were removed and subjected to the plating procedure in order to determine their long-term viabilities in the presence of the various substrate coupons. The procedure is depicted in Fig. 1.

3. Results and discussion

3.1. Syntheses and characterization

The HA monomer prepared by the Bucherer-Berg reaction as described in Section 2.2.1 was chosen for this work because of its three nitrogen atoms which can bind oxidative halogen without the possibility of dehydrohalogenation and because it contains a vinyl group which can easily be copolymerized with a surface-linking monomer. Furthermore, the HA monomer does not homopolymerize under its synthetic conditions, requiring a free radical initiator to do so. The SL monomer was chosen as the linking agent because of its excellent ability to bond to a variety of material surfaces and its methacryl functional group for copolymerization with HA. The ¹H NMR spectra and FTIR spectra for HA, SL, and HASL are shown in Figs. 2 and 3, respectively. Additional ¹H NMR data can be found in the Supporting Information. The key

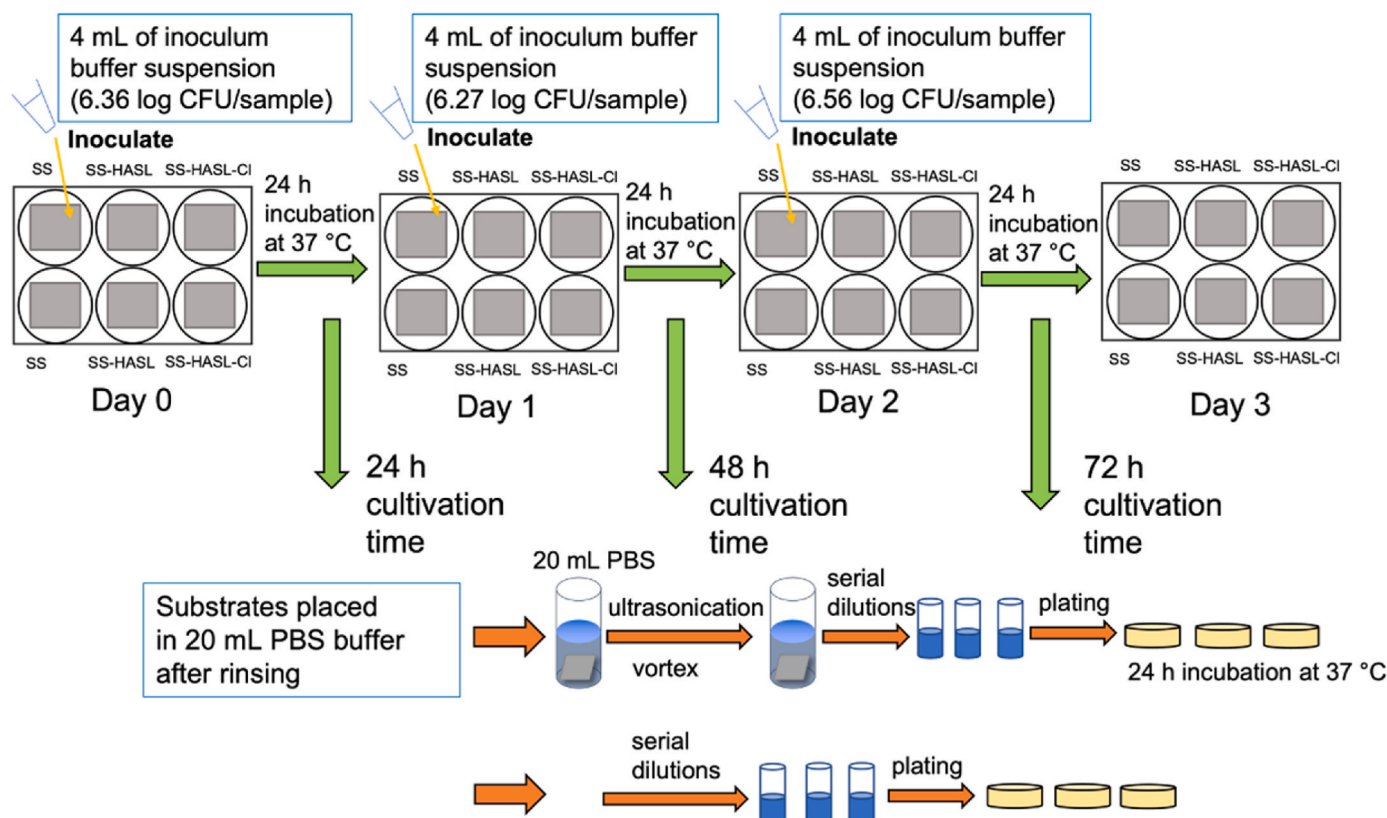


Fig. 1. Procedure employed for biofilm assay.

resonance signals establishing the structure of synthesized HA are assigned to the imide proton (δ 10.57, 1H), the two amide protons (δ 7.80, 1H and δ 7.56, 1H), and the resonances for the vinyl protons (δ 6.19, 1H; δ 5.96, 1H; δ 5.45, 1H) which establishes the fact that the vinyl group survives the synthesis intact. The ^1H NMR of SL provides predominantly resonance signals at δ 3.47, 9H corresponding to the three methoxide groups and δ 1.88, 3H corresponding to the methacryl methyl group, as well as the two expected signals near 6 ppm for the two vinyl protons. Free radical copolymerization of synthesized HA and commercial SL in the presence of AIBN initiator yielded HASL which could be isolated as a white solid exhibiting the ^1H NMR and FTIR spectra shown in Figs. 2 and 3, respectively. The NMR signals due to the imide and amide protons of the HA unit are clearly evident, and the structures for the SL methoxy and methyl groups are also present. The vinyl proton signals are diminished in intensity, but still evident, indicating some unreacted HA and SL units which would be of low concentrations in the HASL coating bath.

The primary bands in the FTIR spectrum of HA are for the carbonyl stretching modes at 1762 and 1708 cm^{-1} . Other data obtained for HA were mp 178 $^{\circ}\text{C}$ (DSC) and molecular weight (239.128 exp; 239.127 calc) from mass spectrometry. The FTIR spectrum of HASL contained bands for the carbonyl units of the hydantoin ring at 1763 and 1710 cm^{-1} and the siloxane group at 1075 cm^{-1} which was also present in the FTIR spectrum of SL at 1078 cm^{-1} . After the HASL deposition procedure described in Section 2.2.5, the ATM-FTIR spectra of SS, SS-HASL, and SS-HASL-Cl were obtained (see Fig. 4).

Vibrational bands at 1767, 1715, 1658, and 1030 cm^{-1} are indicative of the presence of HASL bonded to the SS, and the blue shift in cm^{-1} of the carbonyl bands to 1771 and 1720 cm^{-1} are an indication that chlorination of the surface was successful for SS-HASL-Cl [8]. Further evidence that SS-HASL and SS-HASL-Cl were produced is evident from the X-ray photoelectron spectra (XPS) shown in Fig. 5 below with the elemental compositions presented in Table 2. The N 1s bands in the XPS

of SS-HASL and SS-HASL-Cl are present, but absent for SS, and the Cl 2s and 2p bands for SS-HASL-Cl are present, but absent for SS and SS-HASL, consistent with the expected coatings.

The best means to determine that SS-HASL-Cl is present is to detect oxidative chlorine by an iodometric/thiosulfate titration as will be discussed in the next section.

3.2. Halogen and coating stabilities

After exposing the SS and IN metal coupons to HASL solution (3:1 ethanol:water) and curing for 1 min with a heat gun and then for 30 min in an oven at 130 $^{\circ}\text{C}$ to bond siloxane groups to the metal surfaces, the coupons were chlorinated or brominated as described in Section 2.2.6. Iodometric/thiosulfate titration then provided the results shown in Fig. 6. It is evident that the coupons loaded at least 6×10^{16} atoms of oxidative halogen per cm^2 , which exceeds or equals prior loadings on N-halamine-coated SS [21–25]. Both SS and IN HASL-coated coupons were effective at loading oxidative chlorine, and the acid-etched SS-HASL-Br loaded about 7.5×10^{16} atoms of oxidative bromine per cm^2 ; the unetched IN coupons were not tested for bromination. Prior work in these laboratories [8] and elsewhere [21,25,28] has established that even a loading as low as 5×10^{15} atoms/ cm^2 is sufficient to provide an antimicrobial effect. It should be noted that there was only a very small difference in chlorine loadings for the acid etched and unetched coupons.

This is a very significant result for the HASL coatings. Exposure to a caustic material like piranha solution during pretreatment of SS and IN metals would be a non-starter in an industrial application. To evaluate the coating adhesion stabilities and halogen loading stabilities for the coupons exposed to an agitated aqueous solution, the protocol described in Section 2.2.8 was employed. The results are shown in Fig. 7. While the loss of oxidative chlorine was significant over the time period studied (almost complete by 28 d) due to hydrolysis of the N-Cl moiety, the

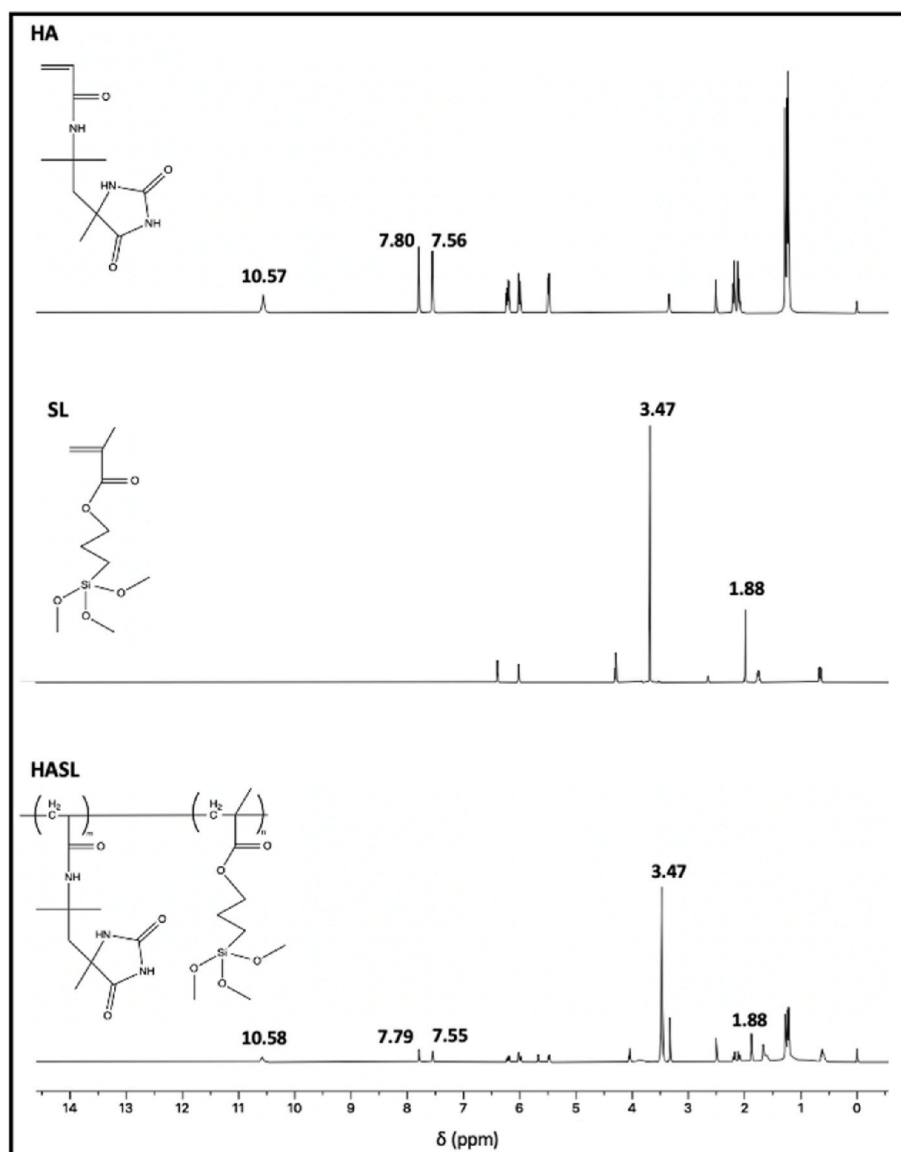


Fig. 2. ^1H NMR (DMSO- d_6) spectra for HA, SL, and HASL.

coupons of both coated metals could be recharged by exposure to dilute free chlorine to approximately the starting loadings. This indicates that the HASL coating itself was not significantly hydrolyzed from the metal surface. Also, none of the coupons were acid etched during pretreatment, further indicating that the caustic acid exposure is not necessary for obtaining SS-HASL and IN-HASL metal coatings. This is a definite advantage of HASL for use in metal coatings which can be rendered antimicrobial by exposure to dilute oxidative free halogen.

3.3. Antibacterial efficacies

The antibacterial efficacy results obtained using the “sandwich test” described in Section 2.2.9 for the native coupons (SS or IN), HASL coated and un-halogenated coupons (SS-HASL and IN-HASL), and HASL coated and halogenated coupons (SS-HASL-X and IN-HASL-X) are presented in Tables 3–6. In some cases, the data refer to coupons both cleaned in solvents and acid-etched, in others only cleaned in solvents. Antimicrobial efficacies of cleaned and acid-etched stainless steel substrates (SS-A-control, SS-A-HASL, and SS-A-HASL-Cl) against *S. aureus* and *E. coli* O157:H7 and are shown in Table 3. Average available chlorine contents of the halogen-activated SS-A-HASL-Cl coupons used for

antimicrobial efficacy analysis were measured as 7.85×10 [16] and 5.83×10 [16] atoms/ cm^2 in exp 1 and exp 2, respectively. All samples (SS-A-control, SS-A-HASL, and SS-A-HASL-Cl) were challenged with *S. aureus* and *E. coli* O157:H7 at approximately 6 logs CFU/sample. As expected, SS-A-control coupons and SS-A-HASL coupons showed a limited degree of bacterial reduction. These observations were in accord with those for N-halamine-modified textile surfaces for which not all viable CFUs could be recovered from an inoculated surface [18]. However, when SS-A-HASL-Cl coupons were exposed to ca. 6 log inocula of *S. aureus* and *E. coli* O157:H7, all of the bacteria, subject to the 50 CFU detection limit, were inactivated within 5–15 min of contact time. The necessary contact times for exp 2 were a bit longer than for exp 1 because of the lower oxidative chlorine loading and higher inoculum populations in exp 2.

Table 4 shows the antimicrobial efficacies of SS-A-control, SS-A-HASL, and SS-A-HASL-Br stainless steel coupons against *S. aureus* and *E. coli* O157:H7. In this test, the SS coupons were acid-etched prior to the coating procedure. Average available bromine contents of the halogen-activated SS-A-HASL-Br coupons used for antimicrobial efficacy analysis were measured as $7.29 \times 10^{*16}$ and $7.53 \times 10^{*16}$ atoms/ cm^2 in exp 1 and exp 2, respectively. The brominated coupons inactivated 6 logs of

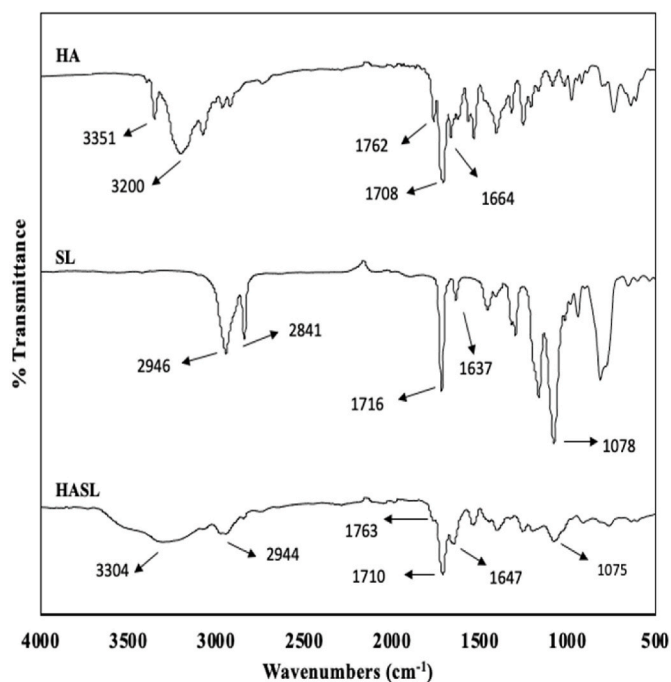


Fig. 3. FTIR spectra for HA, SL, and HASL.

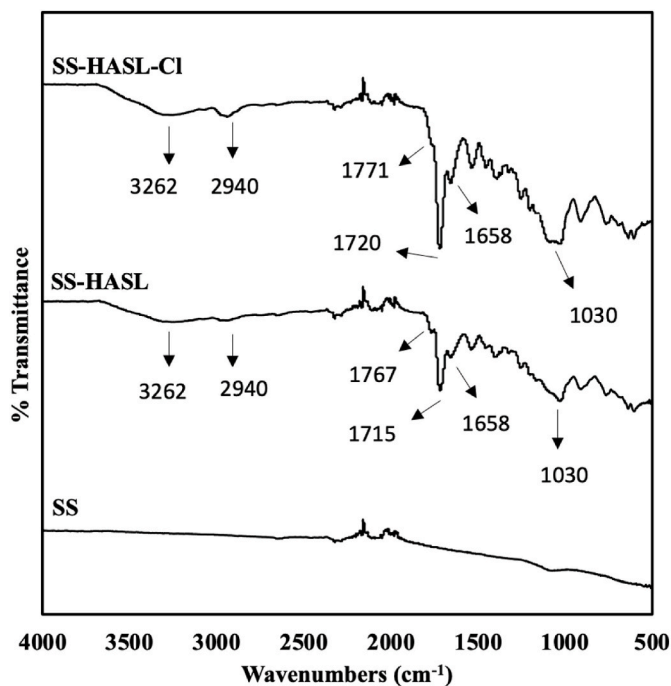


Fig. 4. ATR-FTIR spectra for SS, SS-HASL, and SS-HASL-Cl coupons.

S. aureus and *E. coli* O157:H7 bacteria within 5 min of contact time in both experiments. The brominated surface would be expected to be more bactericidal than an analogous chlorinated one. It was shown in these laboratories many years ago that a combined bromamine is about 50 times more effective than the analogous chloramine against *S. aureus* in aqueous solution [29].

Table 5 provides additional antibacterial efficacy data for SS-C-control, SS-C-HASL, and SS-C-HASL-Cl and SS-C-HASL-Br coupons. For these samples pretreatment consisted of solvent exposure only, i.e. no acid-etching. Upon comparison of the data in Table 5 with those in

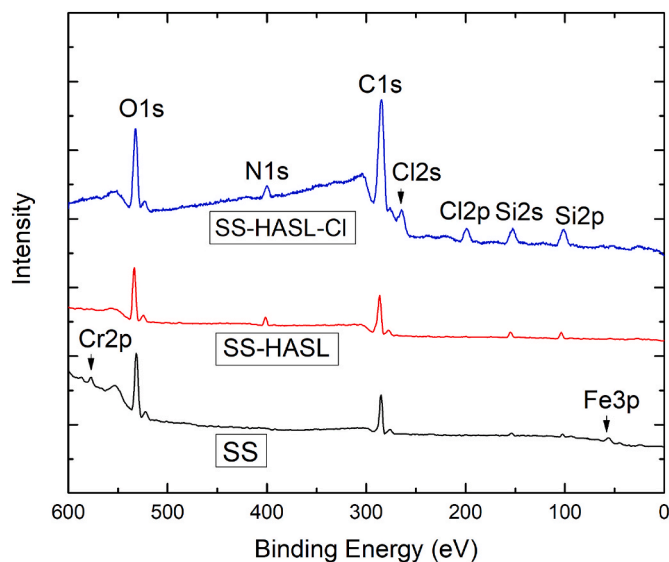


Fig. 5. XPS for the SS, SS-HASL, and SS-HASL-Cl coupons.

Table 2

XPS surface elemental compositions for SS, SS-HASL, and SS-HASL-Cl coupons.

Sample Name	XPS Surface Elemental Composition (at %)						
	C	O	N	Cl	Cr	Si	Fe
SS	57.1	24.2	0.0	NA	1.2	4.4	7.0
SS-HASL	63.6	22.2	5.5	NA	ND	8.7	ND
SS-HASL-Cl	65.2	10.5	2.8	11.1	ND	10.5	ND

NA = not applicable; ND = not determined.

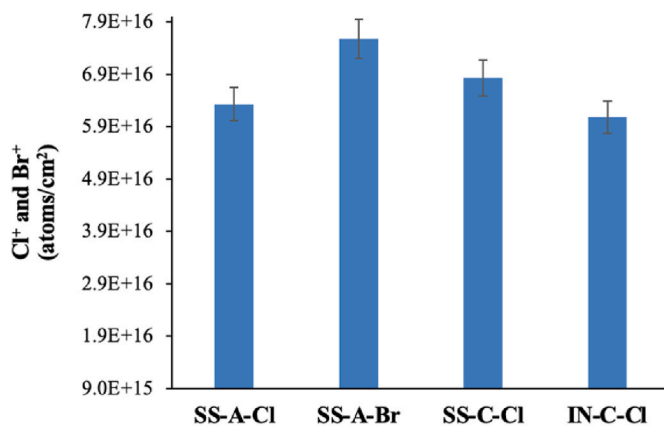


Fig. 6. Oxidative halogen content on the surfaces of the metal coupons after halogenation. SS-A-Cl represents SS-HASL-Cl coupons which were acid etched during pretreatment; SS-C-Cl and IN-C-Cl represent SS-HASL-Cl and IN-HASL-Cl coupons, respectively, which were cleaned with solvents, but not acid etched during pretreatment. Each datum point represents an average of three coated coupons.

Table 3, it is evident that acid-etching led to a somewhat increased oxidative chlorine loading which probably is indicative of increased HASL on the SS surface, although antibacterial efficacy against both bacteria was still observed at longer contact times. Again the brominated surfaces provided complete inactivations in lower contact times than did the chlorinated surfaces even though the oxidative chlorine concentration exceeded that of the oxidative bromine concentration.

Inconel™ coupons that were subjected only to a solvent cleaning process were also coated with HASL copolymer, chlorinated or

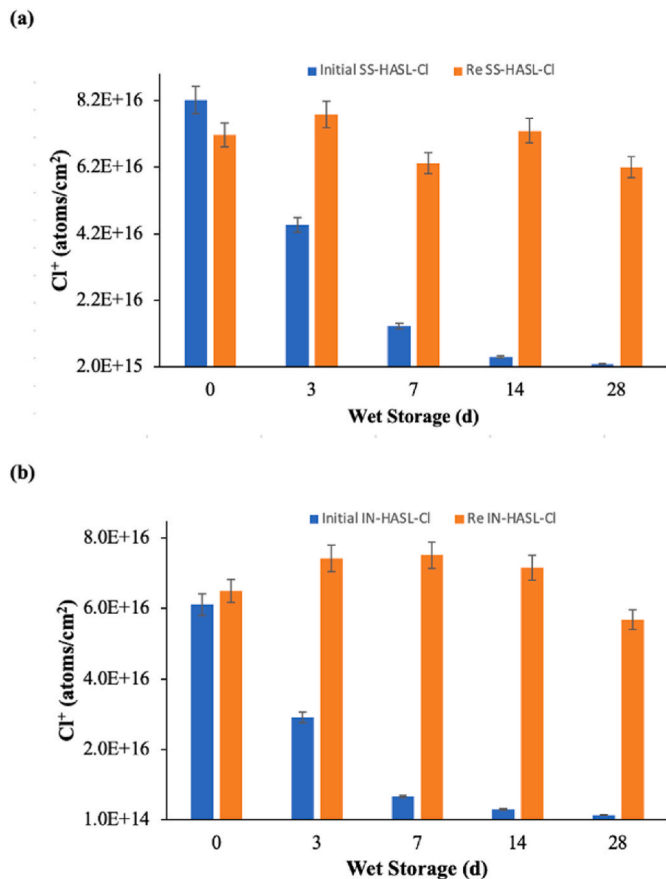


Fig. 7. Coating and oxidative halogen stabilities for cleaned but unetched SS-HASL-Cl and IN-HASL-Cl coupons which were agitated in aqueous detergent solution for various time periods. Re indicates a recharge after reductive titration which removed all oxidative chlorine.

Table 3 Antimicrobial efficacies of acid-etched SS-A-control, SS-A-HASL, and SS-A-HASL-Cl stainless steel coupons against *S. aureus* and *E. coli* O157:H7 bacteria.

Samples	Contact time (min)	Bacteria log CFU reduction			
		<i>S. aureus</i>		<i>E. coli</i> O157:H7	
		Exp1 ^a	Exp2 ^b	Exp1 ^a	Exp2 ^b
Inoculum Population		5.73	7.58	6.04	7.46
SS-A-Control ^a	60	0.32	NA	0.66	NA
SS-A-Control ^b	30	NA	0.81	NA	0.31
SS-A-HASL ^a	60	0.36	NA	0.10	NA
SS-A-HASL ^b	30	NA	0.95	NA	0.19
SS-A-HASL-Cl ^{a,b}	5	5.73	4.61	6.04	3.12
	10	5.73	5.75	6.04	7.46
	15	5.73	7.58	6.04	7.46
	30	5.73	7.58	6.04	7.46
	60	5.73	ND	6.04	ND

NA = not applicable; ND = not determined; the detection limit of bacteria was 50 CFU per sample.

^a Acid-etched SS-A-Control, SS-A-HASL, and SS-A-HASL-Cl were tested against a 5.73 CFU log inoculum of *S. aureus* and a 6.04 CFU log inoculum of *E. coli* O157:H7; oxidative chlorine loading for the SS-A-HASL-Cl was 7.85×10^{16} atoms/cm².

^b Acid-etched SS-A-Control, SS-A-HASL, and SS-A-HASL-Cl were tested against a 7.58 CFU log inoculum of *S. aureus* and a 7.46 CFU log inoculum of *E. coli* O157:H7; oxidative chlorine loading for the SS-A-HASL-Cl was 5.83×10^{16} atoms/cm².

Table 4 Antimicrobial efficacies of acid-etched SS-A-control, SS-A-HASL, and SS-A-HASL-Br stainless steel coupons against *S. aureus* and *E. coli* O157:H7 bacteria.

Samples	Contact time (min)	Bacterial log CFU reduction			
		<i>S. aureus</i>		<i>E. coli</i> O157:H7	
		Exp ^a	Exp ^b	Exp ^a	Exp ^b
Inoculum Population		7.58	6.09	7.46	5.90
SS-A-Control ^a	30	0.81	NA	0.12	NA
SS-A-Control ^b	30	NA	0.02	NA	1.08
SS-A-HASL ^a	30	0.95	NA	0.17	NA
SS-A-HASL ^b	30	NA	0.18	NA	1.33
SS-A-HASL-Br ^{a,b}	5	7.58	6.09	7.46	5.90
	10	7.58	6.09	7.46	5.90
	15	7.58	6.09	7.46	5.90
	30	7.58	6.09	7.46	5.90

NA = not applicable; the detection limit of bacteria was 50 CFU per sample.

^a Acid-etched SS-A-Control, SS-A-HASL, and SS-A-HASL-Br were tested against inocula of 7.58 log CFU *S. aureus* and 7.46 log CFU *E. coli* O157:H7; oxidative bromine loading of the SS-A-HASL-Br was 7.29×10^{16} atoms/cm².

^b Acid-etched SS-A-Control, SS-A-HASL, and SS-A-HASL-Br were tested against inocula of 6.09 log CFU *S. aureus* and 5.90 log CFU *E. coli* O157:H7; oxidative bromine loading of the SS-A-HASL-Br was 7.53×10^{16} atoms/cm².

Table 5 Antimicrobial efficacies of cleaned, but not acid-etched, SS-C-Control, SS-C-HASL, and SS-C-HASL-X stainless steel coupons against *S. aureus* and *E. coli* O157:H7 bacteria.

Samples	Contact time (min)	Bacterial log CFU reduction			
		<i>S. aureus</i>		<i>E. coli</i> O157:H7	
		Exp ^a	Exp ^b	Exp ^a	Exp ^b
Inoculum Population		5.99	5.94	6.32	6.19
SS-C-Control ^a	30	0.09	NA	0.10	NA
SS-C-Control ^b	30	NA	0.29	NA	1.28
SS-C-HASL ^a	30	0.05	NA	0.03	NA
SS-C-HASL ^b	30	NA	0.21	NA	0.03
SS-C-HASL-X ^{a,b}	5	2.55	5.94	0.96	1.57
	10	2.73	5.94	1.03	6.19
	15	5.99	5.94	1.89	6.19
	30	5.99	5.94	6.32	6.19

NA = not applicable; the detection limit of bacteria was 50 CFU per sample.

^a Un-etched SS-C-Control, SS-C-HASL, and SS-C-HASL-Cl were tested against inocula of 5.99 log CFU *S. aureus* and 6.32 log CFU *E. coli* O157:H7; oxidative chlorine loading of the SS-C-HASL-Cl was 2.48×10^{16} atoms/cm².

^b Un-etched SS-C-Control, SS-C-HASL, and SS-C-HASL-Br were tested against inocula of 5.94 log CFU *S. aureus* and 6.19 log CFU *E. coli* O157:H7; oxidative bromine loading of the SS-C-HASL-Br was 9.72×10^{15} atoms/cm².

brominated, and tested for antibacterial efficacy against *S. aureus* and *E. coli* O157:H7. The results for IN-C-Control, IN-C-HASL, and IN-C-HASL-Cl and IN-C-HASL-Br are presented in Table 6. Chlorinated Inconel™ coupons (IN-HASL-Cl) inactivated 6 logs of *S. aureus* and *E. coli* bacteria within 5 min contact time in Experiment (a). The contact time for IN-HASL-Cl in Experiment (b) was 15–30 min. The longer contact time was necessary because of a much lower loading of oxidative chlorine in Experiment (b) which was effected by design to test the efficacies of the coated substrate having different chlorine loadings. Brominated IN coupons (IN-HASL-Br) gave a complete inactivation of both bacteria in experiment (c) within 5–10 min. As expected, bromine was more effective as an antimicrobial than was chlorine as noted for SS.

Thus, it has been found that both chlorinated and brominated HASL coatings on stainless steel and Inconel™ are effective in inactivating Gram-positive and Gram-negative bacteria on their surfaces in a few minutes of contact. The pretreatment of the SS with piranha solution

Table 6

Antimicrobial efficacies of cleaned, but not acid-etched, IN-C-control, IN-C-HASL, and IN-C-HASL-X Inconel™ coupons against *S. aureus* and *E. coli* O157:H7 bacteria.

Samples	Contact time (min)	Bacterial log CFU reduction					
		<i>S. aureus</i>			<i>E. coli</i> O157:H7		
		Exp ^a	Exp ^b	Exp ^c	Exp ^a	Exp ^b	Exp ^c
Inoculum Population		6.09	5.96	5.94	5.90	6.32	6.19
IN-C-Control ^a	30	0.01	NA	NA	0.05	NA	NA
IN-C-Control ^b	30	NA	0.03	NA	NA	0.17	NA
IN-C-Control ^c	30	NA	NA	0.07	NA	NA	0.28
IN-C-HASL ^a	30	0.12	NA	NA	0.56	NA	NA
IN-C-HASL ^b	30	NA	0.11	NA	NA	0.35	NA
IN-C-HASL ^c	30	NA	NA	0.01	NA	NA	0.04
IN-C-HASL-X ^{a,b,c}	5	6.09	2.04	5.94	5.90	2.29	3.04
	10	6.09	3.08	5.94	5.90	2.81	6.19
	15	6.09	5.96	5.94	5.90	3.29	6.19
	30	6.09	5.96	5.94	5.90	6.32	6.19

N = not applicable; the detection limit of bacteria was 50 CFU per sample.

^a Un-etched IN-C-Control, IN-C-HASL, and IN-C-HASL-Cl were tested against inocula of 6.09 log CFU *S. aureus* and 5.90 log CFU *E. coli* O157:H7; oxidative chlorine loading of the SS-C-HASL-Cl was 6.05×10^{16} atoms/cm².

^b Un-etched IN-C-Control, IN-C-HASL, and IN-C-HASL-Cl were tested against inocula of 5.96 log CFU *S. aureus* and 6.32 log CFU *E. coli* O157:H7; oxidative chlorine loading of the SS-C-HASL-Cl was 1.97×10^{16} atoms/cm².

^c Un-etched IN-C-Control, IN-C-HASL, and IN-C-HASL-Br were tested against inocula of 5.94 log CFU *S. aureus* and 6.19 log CFU *E. coli* O157:H7; oxidative bromine loading of the IN-C-HASL-Br was 1.02×10^{16} atoms/cm².

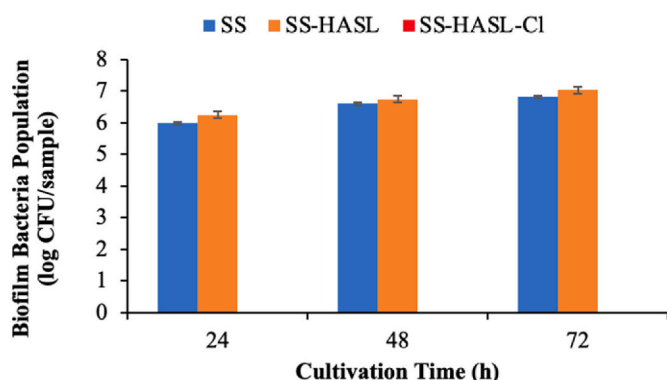


Fig. 8. Bacterial biofilm formation by adhesion of *P. aeruginosa* on cleaned, unetched stainless steel; each datum represents an average of quadruplicate determinations. The detection limit of bacteria was 200 CFU per sample.

does provide increased HASL bonding sites relative to simple cleaning with solvents, but in our opinion, cleaning SS or IN surfaces with solvents should be sufficient to create a satisfactory antimicrobial surface when HASL is the coating polymer.

3.4. Biofilm assays

Quantitative measurements of bacterial attachment and biofilm formation on the cleaned, un-etched SS, SS-HASL, and SS-HASL-Cl coupons as described in Section 2.2.10 were performed. Each coupon was inoculated with 6.57 logs of CFU/sample of *P. aeruginosa* suspension (3 mL) on day 0 and incubated for 24, 48, and 72 h at 37 °C. Quadruplicate runs were performed. Viable bacterial cells adhered onto the surface of the substrates after the several cultivation times were enumerated. Viable bacteria collected after each cultivation time represented biofilm accumulation occurring due to both growth and attachment onto the substrate surfaces. The results are presented in

Fig. 8. Biofilm growth was observed on the surface of the sterilized, uncoated SS coupons, for which the viable bacteria count was increased from 6.00 log CFU/sample after 24 h to 6.82 log CFU/sample after 72 h of cultivation time.

As expected, a similar trend (6.24–7.02 log CFU/sample) was observed for sterilized HASL coated coupons for which HASL treatment without chlorine activation was unable to prevent biofilm formation on SS-HASL substrates since the substrates held no antimicrobial activity. In contrast, there was no viable bacterial growth observed for HASL coated and chlorinated coupons (SS-C-HASL-Cl) after 24, 48, or 72 h of cultivation time (Fig. 8). This result indicated that biofilm growth on the surface of SS-HASL-Cl was prevented and showed that halogenated substrates can inhibit bacterial adhesion and proliferation. This result is attributed to the antimicrobial efficacies of the halogenated substrates as shown in Table 5.

Given that it was possible that all of the bacteria in the solution above the coupons were inactivated rapidly upon kinetic contact with the surface of the SS-C-HASL-Cl coupon leaving no challenge bacteria to create biofilm formation on the coupon after 24 h cultivation, the other experiment described for biofilm formation in Section 2.2.11 was performed in quadruplicate. After each cultivation time, an aliquot of suspension over each coupon was removed and assayed for viable colonies before old inoculum was replaced by new inoculum. The bacterial populations used for the three different contact times were 2.27×10^6 (6.36 log), 1.87×10^6 (6.27 log), and 3.60×10^6 (6.56 log) CFU/sample for the 24, 48, and 72 h contact times, respectively. The results are shown in Fig. 9(a). As in the previous experiment, the bacterial population contacting the SS coupons and SS-HASL coupons increased after

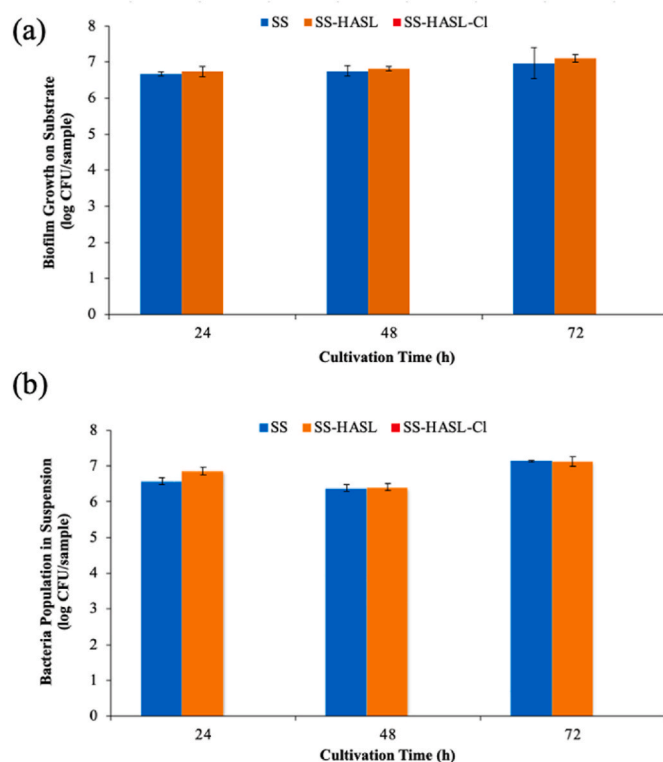


Fig. 9. Bacterial adhesion onto substrate (a), and viable bacteria population in inoculated buffer suspension (b); each coupon was inoculated with 6.36 log CFU on day 0 and incubated for 24 h. Then each coupon was inoculated for a second time with 6.27 log CFU (freshly prepared) on day 1 and incubated for an additional of 24 h; the total incubation time was 48 h. Then each coupon was inoculated for a third time with 6.56 log CFU on day 2 and incubated for an additional 24 h; the total incubation time was 72 h. Each datum represents an average of quadruplicate determinations. The detection limit of bacteria was 200 CFU per sample.

72 h of cultivation to 6.96 log CFU/sample and 7.10 log CFU/sample, respectively, indicating the establishment of biofilms for each. However, there were no viable CFU recovered from the SS-HASL-Cl coupons after any of the cultivation times. Thus SS-HASL-Cl was effective at preventing biofilm formation for at least 72 h after total exposure to 7.74×10^6 (6.89 log) CFU/sample. Fig. 9(b) shows the results for the remaining CFU/sample in the suspensions above the coupons at the three cultivation times. The suspension above the SS and SS-HASL coupons contained an average of 7.14 log CFU/sample and 7.13 log CFU/sample, respectively, after 72 h cultivation, indicating no inactivation by these two substrates within the suspensions. On the other hand, there were no viable CFU contained above the SS-HASL-Cl coupons at any time. This shows that the chlorinated samples were not only

able to prevent biofilm formation, but they also inactivated the bacteria contained in the suspensions above them upon contact. The results from these antimicrobial contact and biofilm experiments provide evidence that there is potential for use of SS-HASL-Cl on substrate surfaces which contact water in spacecraft and other applications. Although IN-HASL-Cl, SS-HASL-Br, and IN-HASL-Br coupons were not subjected to the biofilm assays, it is likely that they too would be effective in the applications.

4. Conclusions

It has been demonstrated that the substrates stainless steel and Inconel™ used in water storage systems of spacecraft can be coated with the copolymer HASL developed in these laboratories. Upon exposure to dilute solutions of chlorine or bromine, the coating becomes charged with oxidative halogen. The coatings are then effective in inactivation of bacterial pathogens such as *Staphylococcus aureus* and *Escherichia coli* O157:H7. They also were able to prevent *Pseudomonas aeruginosa* biofilm formation. The halogenated HASL polymer coating does lose halogen slowly over time upon exposure to water through hydrolysis. However, the HASL coating itself is stable on exposure to water, and halogen can be recharged on the polymer coating by exposure to dilute chlorine or bromine. Halogen recharge will be necessary for continuous inhibition of biofilm formation over an extended time period. Recharge of halogen onto the HASL coating is compatible with earth-bound water handling systems, but a process suitable for use on the spacecraft would need to be developed in future work, as well as assessment of the long-term (multiple months or years) stability of the HASL coating.

Declare of competing interest

Notes, The authors declare no competing financial interest.

CRediT authorship contribution statement

Buket Demir: Data curation, Formal analysis, Writing – original draft, Writing – review & editing, Review & Editing. **Alicia Taylor:** Data curation, Formal analysis, Writing – original draft, Writing – review & editing, Review & Editing. **R.M. Broughton:** Funding acquisition, Conceptualization, Project administration, Supervision, Formal analysis, Writing – review & editing. **T.-S. Huang:** Data curation, Formal analysis, Writing – review & editing. **M.J. Bozack:** Data curation, Formal analysis, Writing – review & editing. **S.D. Worley:** Conceptualization, Project administration, Supervision, Formal analysis, Writing – original draft, Writing – review & editing.

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Supporting information

¹H NMR Spectra for compounds HA, SL, and HASL.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.biofilm.2022.100076>.

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