Self-healing antimicrobial polymer coating with efficacy in the presence of organic matter

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ABSTRACT

A method to prepare a self-healing, antimicrobial polymer coating that retains efficacy against *Escherichia coli* O157:H7 in the presence of organic matter is reported. A coating composed of branched polyethyleneimine (PEI) and styrene maleic anhydride copolymer (SMA) was applied to a maleic anhydride functionalized polypropylene support. The chemistry of the polymer coating was designed to impart hydrophobicity due to the styrene subunits, intrinsic antimicrobial character (>99.9% reduction) from the cationic primary amine groups, and enhanced antimicrobial character (>99.99% reduction) after chlorination of *N*-halamine forming groups. Antimicrobial effectiveness was demonstrated under conditions of increasing organic load. Up to 500 ppm horse serum, chlorinated coatings retained full antimicrobial character (>99.99% reduction). Even at 50,000 ppm of horse serum, the coating provided ~ 90% reduction as prepared, and between ~ 75% and ~ 80% reduction in the form of *N*-halamines. Microscopy confirmed no evidence of bacterial adhesion on the coating surface. Finally, the coating exhibited self-healing properties after exposure to acid and alkaline solutions and restoration by heat, as confirmed through spectroscopy from the rebuilding of characteristic chemical bonds. Such robust antimicrobial polymer coatings with efficacy under conditions of increasing organic load may support reducing microbial cross-contamination in food and biomedical industries.

KEYWORDS

Antimicrobial material, *N*-halamines, polycations, self-healing material, antimicrobial coating, microbial cross-contamination.

35 Graphical Abstract

36

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1 INTRODUCTION

Microbial contamination of surfaces and materials remains a problem in the food and biomedical industries both to public health and as a financial burden to the government. It is estimated that every year in The United States of America approximately 48 million people get sick from foodborne diseases [1]. In addition, in 2011 there were approximately 722,000 health-care related infections [2]. According to the Food and Drug Administration of The United States of America, contaminated utensils and surfaces are among the top 5 risk factors for foodborne outbreaks [3]. To decrease the risk of microbial infections, the food and biomedical industries employ mainly physical and chemical sanitization methods that involve the use of heat (in the form of steam, irradiation, etc.) or disinfectants like quaternary ammonium compounds or hydrogen peroxide [4]. However, such sanitization methods suffer limitations such as reduced efficacy in the presence of organic matter (almost unavoidable in food processing equipment and medical utensils), degradation of surfaces with repeat sanitization (resulting in a rough morphology more supportive of biofilm establishment), and development of microbial resistance [5]. The development of antimicrobial materials and coatings therefore remains a promising opportunity to support cleaning and sanitization protocols in preventing microbial contamination in the food and biomedical industries.

One strategy to prepare antimicrobial materials has been the application of thin coatings with antimicrobial character onto the surface of commonly used materials [5-7]. One of the main advantages of this concept is to keep the bulk properties of the coated materials intact [8]. Antimicrobial character can be imparted by embedding antimicrobial agents throughout the coating matrix [9], however this strategy requires leaching of the antimicrobial to be effective, resulting in eventual loss in antimicrobial activity and the potential for development of resistant

organisms [10-12]. Integration of antimicrobial moieties (e.g. *N*-halamines, cationic) into polymer structures seeks to overcome these limitations. *N*-halamines are a class of antimicrobials in which nitrogen atoms bond with halogens (most commonly chlorine), exert antimicrobial character by releasing the halogen, and are able to regenerate antimicrobial character by repeated exposure to the halogen source for many cycles of disinfection. This regenerative property constitutes the main feature of *N*-halamines, making them able to be reused constantly [13-16]. The mechanisms involved in the microbial inactivation caused by *N*-halamines have not been confirmed, but are believed to involve damage of genetic information, cell membrane disruption, and denaturation of vital biomolecules [9, 17]. *N*-halamines have shown efficacy against several types of microorganisms in a number of published studies, but have limited efficacy in the presence of organic matter and may not present sufficient durability due to the necessary repeated exposure to a halogen source, as well as other deteriorating agents (e.g. light, heat, pH extremes) [9, 18-21]. Cationic polymers are another classification of antimicrobials believed to inactivate microorganisms by membrane disruption [9, 22, 23]. Like *N*-halamines, their effectiveness has been demonstrated against several microorganisms [24-27]; however, given their charged nature, they can be prone to fouling [24, 28-30], and have been reported to lose biocidal efficacy in the presence of organic matter [9, 31]. Prior publications indicate that integration of dual biocidal character in a coating can improve antimicrobial efficacy without the need for frequent rechlorination [20, 32-34], yet stability of the coatings and preservation of antimicrobial efficacy in the presence of organic matter remains a significant challenge [9].

We report a method for coat-cure preparation of a polymer coating which exhibits antimicrobial efficacy due to integration of both *N*-halamine and cationic moieties within the

polymer structure. The coating did not promote microbial adhesion, despite presence of antimicrobial polycations. Preparation of the coating by assembly of polymers which generate reactive chemical species (e.g. amine, carboxylic acids, carbonyls, anhydride) after damage resulted in a coating with the molecular mobility and thermodynamic reversibility necessary to enable self-healing through application of heat [21, 35-38]. The antimicrobial coating was applied to a reactive blend composed of polypropylene and polypropylene-*graft*-maleic anhydride, and its effectiveness was demonstrated under increasing levels of organic matter against the pathogen *Escherichia coli* O157:H7.

2 Materials and Methods

Polypropylene (PP, isotactic) and styrene maleic anhydride copolymer (SMA, 6 kDa) were purchased from Scientific Polymer Products (Ontario, NY, USA). Polypropylene-*graft*-maleic anhydride (PP-g-MA, ~ 9.1 kDa), branched polyethyleneimine (PEI, 25 kDa), 4-(2-hydroxyethyl)-1-piperazineethane-sulfonic acid (HEPES), and horse serum were purchased from Sigma-Aldrich (St. Louis, MO, USA). The dye Orange (II) (Cert) (AO7), sodium hypochlorite solution (5% chlorine), and reagent grade ethanol were purchased from Acros Organics (Fair Lawn, NJ, USA). *N,N*-Diethyl-*p*-phenylenediamine (DPD) total chlorine reagent powder was purchased from Hach Co. (Loveland, CO, USA). Tryptic soy agar (TSA), tryptic soy broth (TSA) and neutralizing buffer (containing sodium thiosulfate to neutralize residual chlorine) were purchased from Difco, Becton Dickinson (Sparks, MD, USA.)

2.1 Preparation of antimicrobial coating

Pellets of PP were cleaned under sonication first with isopropanol, then with acetone, and then with deionized (DI) water (two cycles of 10 min for each solvent). Pellets of PP-g-MA were

106 cleaned under sonication first with acetone and then with DI water (two cycles of 10 min for
107 each solvent). Cleaned pellets were left drying overnight under anhydrous calcium sulfate (RH <
108 20%). Cleaned pellets of PP (90% w/w) and PP-g-MA (10% w/w) were blended in a Brabender
109 mixer model R.E.E.6. (50 mL capacity, Duisburg, Germany) at 190 °C and 60 rpm for 5 min.
110 Then, cooled pieces of the blend (PP/PP-g-MA) were hot-pressed at the same temperature with a
111 load force of 9000 lbs. to a final thickness of 0.24 ± 0.03 mm. The hot-pressed films were cut
112 into 2×2 cm coupons, cleaned with acetone and DI water as described previously, and left
113 drying overnight under the same conditions.

114 The antimicrobial polymer coating was applied to pressed films of PP/PP-g-MA by sequential
115 application of PEI and SMA as illustrated in Figure 1. PEI and SMA were separately mixed with
116 acetone at concentrations of 0.06 g mL^{-1} and 0.04 g mL^{-1} , respectively, and sonicated for 20 min
117 or until the polymers were completely dissolved. Aliquots of each polymer solution (300 μL)
118 were spin coated onto PP/PP-g-MA coupons for 1 min at 3000 rpm in the order: PEI, SMA, PEI.
119 Coated coupons were cured for 20 min at 165 °C to enable crosslinking between amines and
120 anhydride groups. Cured, coated coupons were labeled as PP-PEI-SMA-PEI.

Figure 1. Preparation of the antimicrobial coating.

2.2 Antimicrobial coating characterization

2.2.1 Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy (ATR-FTIR). At every step of the preparation of the antimicrobial coating, coupons were analyzed with an IRTracer-100 spectrometer (Shimadzu corp., Japan), equipped with a diamond ATR crystal (penetration depth $\sim 2 \mu\text{m}$). Multiple spots of coupons from each of at least 3 separately prepared sets (3 replicates) were analyzed using Happ-Genzel apodization (32 scans per spot, with a resolution of 4 cm^{-1}). Obtained spectra were subjected to baseline correction and analyzed with the software KnowItAll (Bio-Rad Laboratories, Philadelphia, PA, USA). Average spectra from

each preparation step were selected and combined for comparison. After the self-healing studies (explained below), coupons were analyzed under the same conditions.

2.2.2 Primary amine quantification. The Acid Orange 7 (AO7) assay [40] was employed to determine the amount of primary amines on the surface of the antimicrobial coating. The 2×2 cm PP-PEI-SMA-PEI coupons were cut into 1×1 cm pieces and individually immersed in test tubes containing 5 mL of a 1 mM solution of AO7, adjusted with HCl to a pH of 3.0, and shaken for 3 h. Then, coupons were rinsed with copious pH 3.0 DI water to desorb unbound dye, immersed individually in test tubes containing 5 mL of pH 12 DI water adjusted with NaOH, and shaken for 15 min to desorb bound dye. Absorbances were read at 455 nm and the primary amine content was determined by comparison to a standard curve prepared with pH 12 solutions containing different concentrations of AO7. This evaluation was conducted with PP-PEI-SMA-PEI coupons obtained from separately prepared sets (3 replicates, with at least two 1×1 cm coupons per replicate). PP/PP-g-MA coupons were analyzed under the same conditions as control.

2.2.3 N-halamine quantification. N-halamine forming groups were chlorinated by immersing PP-PEI-SMA-PEI coupons (1×1 cm pieces) individually in test tubes containing 1 mL of 200 ppm of chlorine prepared from sodium hypochlorite solution (5% chlorine, confirmed through iodometric titration [41]). To characterize chlorination kinetics, PP-PEI-SMA-PEI coupons were removed from chlorine solution at different time points, rinsed with copious DI water, and analyzed for N-halamine content using the colorimetric DPD assay [12, 20, 42, 43]. Briefly, 50 μ L DPD reagent (prepared by dissolving individual packets of DPD total chlorine reagent in 1 mL DI water) was added to a test tube containing 2 mL of DI water and the PP-PEI-SMA-PEI coupons, and shaken for 5 min for color generation. Absorbances were read at 512 nm and the N-

halamine content determined by comparison to a standard curve of aqueous chlorine solution. To determine the time to reach chlorine saturation, the exponential association model was used:

$$NH = NH_{\text{asympt}}(1 - e^{-Kt}) \quad (1)$$

where NH is the N -halamine content (nmol cm^{-2}), NH_{asympt} is the theoretical maximum amount of N -halamines reached at saturation, K is the kinetic factor (min^{-1}), and t is the time (min). N -halamine values at the different time points were fitted with Equation 1 using GraphPad Prism 5 version 5.04 (GraphPad Software Inc., La Jolla, CA, USA). This evaluation was performed 3 times with at least 2 coupons for each time point prepared from separately prepared sets (3 replicates). As controls, PP/PP-g-MA and unchlorinated PP-PEI-SMA-PEI coupons were used.

2.2.4 Atomic Force Microscopy (AFM). Surface topographies of PP/PP-g-MA and PP-PEI-SMA-PEI coupons were analyzed with a Dimension 3100 atomic force microscope (Digital Instruments, Santa Barbara, CA, USA) with tapping mode using an uncoated Si N-type tip ($f_0 = 200 - 400 \text{ kHz}$, Applied NanoStructures Inc., Mountain View, CA, USA). At least 3 measurements were taken, and mean square roughness (R_q) values were obtained from the SPIP 6.0.6 software (Scanning Probe Image Processor, Image Metrology, Hørsholm, Denmark).

2.2.5 Water contact angle and surface pK_a . Advancing (θ_A) and receding (θ_R) water contact angles were determined with a drop shape analyzer DSA100 (Krüss, Hamburg, Germany). PP/PP-g-MA and PP-PEI-SMA-PEI coupons were cut into $1 \times 2 \text{ cm}$ samples for analysis. At least two spots were analyzed for each $1 \times 2 \text{ cm}$ coupon by applying a $5 \mu\text{L}$ drop of DI water at $25 \mu\text{L min}^{-1}$. Data were obtained with the DSA software version 1.91.0.2 (Krüss, Hamburg, Germany). For the surface pK_a determination, the static contact angle (θ_s) at different pH levels

was determined as follows [20, 44-46]. Values of θ_s were obtained by applying 5 μ L of 10 mM HEPES, adjusted to pH 1 – 13 by HCl and NaOH on PP/PP-g-MA and PP-PEI-SMA-PEI coupons and measuring the contact angle after 15 s of application with the DSA software (1 $\times$ 2 cm coupons, at least 2 spots per coupon). At least one 1 $\times$ 2 cm coupon from each of 3 separately prepared sets was used for contact angle and pK_a determinations (at least 3 replicates).

2.3 Antimicrobial evaluations

A loopful of frozen culture of *Escherichia coli* O157:H7 ATCC 43895 (kept at – 80 °C in 25% glycerol, provided by Dr. Lynne A. McLandsborough from the food science department at the University of Massachusetts Amherst, USA) was inoculated onto TSA and incubated at 37 °C for 24 h. Then, an individual colony from the TSA plates was inoculated in 9 mL of TSB and incubated for 14 h at the same temperature. A loopful of the overnight broth was inoculated onto TSA and incubated at 37 °C for 24 h. The resulting colonies on TSA were kept at refrigeration temperature for no more than 3 weeks for use in preparing inocula as described below.

For the antimicrobial evaluations, a single colony of *E. coli* O157:H7 was inoculated in TSB and incubated for 14 h at 37 °C. A 1% dilution of the overnight culture was prepared in fresh TSB and incubated at 37 °C to mid-exponential phase. Finally, a 0.1% aqueous dilution of the mid-exponential phase culture was prepared for a microbial load of $\sim 6 \log(\text{CFU mL}^{-1})$. Horse serum, previously heat inactivated at 56 °C as suggested by the provider, was added to the aqueous suspension at concentrations up to 50,000 ppm to demonstrate the efficacy of the antimicrobial material in the presence of organic matter [47]. For each treatment, four 1 $\times$ 1 cm coupons were put in contact with 1 mL of bacterial suspension and rotated (60 rpm) for 2 h at 32 °C [20, 21, 43]. The initial 10% serial dilution was prepared with neutralizing buffer (containing

sodium thiosulfate) to quench chlorine, and subsequent 10% serial dilutions were prepared with 0.9% saline water. To lower the limit of detection to 1 log(CFU mL⁻¹), the entire volume (1 mL) of the first dilution was plated onto 3 TSA plates (333 µL per plate). TSA plates were incubated for 48 h at 37 °C, and colonies were counted thereafter with a plate reader Scan 500 (Interscience, Saint-Nonla-Brèteche, France). As negative controls, bacterial suspensions alone, PP/PP-g-MA, and chlorinated PP/PP-g-MA (chlorinated for 1 h under the same scheme explained before for the *N*-halamine quantification) were used. As a positive control, diluted bleach was added to the bacterial suspensions to imitate the chlorine content given by the four 1 × 1 cm coupons of chlorinated PP-PEI-SMA-PEI. The other 2 treatments were PP-PEI-SMA-PEI and chlorinated PP-PEI-SMA-PEI (coupons were chlorinated for 1 h under the scheme described previously for the *N*-halamine quantification). The time for chlorination was chosen because this was the time required to reach *N*-halamine saturation, as it will be explained below.

SEM was used to confirm lack of bacterial attachment on the antimicrobial coating. PP-PEI-SMA-PEI coupons with 0 and 50000 ppm horse serum, with or without chlorination were evaluated. After the antimicrobial efficacy assay, coupons were rinsed 3 times with sterile DI water and then immersed for 45 s in reagent grade ethanol to fix any bound bacteria [48]. Then, coupons were sputter-coated with gold for 30 s under argon with a Cressington Sputter Coater 108 Auto (Ted Pella, Inc., Redding, CA, USA). At least 10 images were acquired across each coupon using a scanning electron microscope (JCM-6000 NeoScope, JEOL, Japan) at 10 kV.

2.4 Self-healing evaluations

PP-PEI-SMA-PEI coupons were characterized for stability against chemical degradation and capacity for self-healing. Resistance of the coatings to degradation was tested using an

adaptation of standard conditions in which coupons are submerged in either 5% HCl or 4% NaOH with rotation at 20 °C for 10 hours [49-51]. After rinsing with copious DI water and drying under filtered air, coupons were subjected to a self-healing treatment of heating at 140 °C for 20 min. Coupons were analyzed by SEM, ATR-FTIR, *N*-halamine content, and X-Ray Photoelectron Spectroscopy (XPS) at each stage (as prepared, either HCl or NaOH treated, and after self-healing at 140 °C). For XPS analysis, at least 1 coupon from each of 3 separately prepared sets (3 replicates) was analyzed at each stage with a Physical Electronics Quantum 2000 (Physical Electronics, Chanhassen, MN, USA) at an angle of 45° with Al K α excitation (with a spot size of 100 μ m and penetration depth of 2.5 – 3.0 nm). Survey scans were collected at a pass energy of 187.85 eV (step size of 1.6 eV), and high resolution scans of O 1s and N 1s were collected at a pass energy of 46.95 eV (step size of 0.4 eV). Results were analyzed with the software Multipak version 6.1A (Physical Electronics, Chanhassen, MN, USA), using the Gaussian-Lorentzian model (90% Gaussian).

2.5 Statistical analysis

When appropriate, significant differences between treatments were determined through analysis of variance followed by Tukey's pairwise comparisons with a 95% confidence interval using the software GraphPad Prism 5 version 5.04 (GraphPad Software Inc., La Jolla, CA, USA).

3 RESULTS AND DISCUSSION

3.1 Antimicrobial coating characterization

ATR-FTIR spectra of the polymers used to prepare PP/PP-g-MA solid supports (Figure 2, top) revealed absorbance bands characteristic of anhydrides (C=O vibrations at 1850 cm⁻¹ and 1780

cm⁻¹), as well as the carbonyl band from carboxylic acids at 1740 – 1720 cm⁻¹. It can be observed that after blending, these absorbances become less pronounced. The bottom graph shows the steps during the preparation of the antimicrobial coating: (A) PP/PP-g-MA, (B) application of first layer of PEI, (C) application of the SMA layer, (D) application of the second layer of PEI, and (E) curing. The band at ~ 1550 cm⁻¹ in spectrum B (characteristic of the N–H bond in-plane bend of secondary amides) suggests some initial binding between the primary amine groups of PEI and the maleic anhydride groups on the surface of PP/PP-g-MA. Spectrum C shows the characteristic bands of anhydrides at 1850 cm⁻¹ and 1780 cm⁻¹ from SMA. After the application of the second layer of PEI (spectrum D), the disappearance of the bands at 1850 cm⁻¹ and 1780 cm⁻¹ and the increment in the absorbance level at ~ 1550 cm⁻¹ suggests opening of all the maleic anhydride rings of SMA and higher crosslinking (amide bond formation) between PEI and SMA. Finally, a more pronounced absorbance at ~ 1650 cm⁻¹ (corresponding to the C=O group of amides and imides) suggests the ring-closing step brought by curing at 165 °C (Figure 1) [20, 52].

Figure 2. ATR-FTIR spectra of the raw materials to obtain PP/PP-g-MA (top) and the preparation steps of PP-PEI-SMA-PEI.

AO7 and DPD dye assays were used to quantify the primary amine and *N*-halamine content, respectively, of PP-PEI-SMA-PEI. As expected, no measurable primary amines were detected on the surface of PP/PP-g-MA, while PP-PEI-SMA-PEI coupons exhibited 334.2 ± 33.0 nmol primary amines per cm^2 . Figure 3 shows the exponential association curve (Equation 1)

illustrating the chlorination kinetics and chlorine holding capacity of PP-PEI-SMA-PEI. *N*-halamine saturation was reached after 1 h of exposure to 200 ppm of chlorine ($NH_{\text{asympt}} = 120.2 \pm 3.9 \text{ nmol cm}^{-2}$, $K = 0.01 \pm 0.0 \text{ min}^{-1}$). No measurable chlorine was detected from PP/PP-g-MA coupons that had been exposed to equivalent chlorination conditions (up to 120 min under 200 ppm of chlorine). These results support the findings of FTIR analysis which suggest that the PEI-SMA-PEI coating introduced both cationic and *N*-halamine forming antimicrobial moieties.

Figure 3. *N*-halamine (vertical axis, NH) quantification. Data fitted with Equation 1 (trend line, $R^2 = 0.94 \pm 0.01$).

Figure 4 shows the AFM images of PP/PP-g-MA (top) and PP-PEI-SMA-PEI (bottom). The R_q values of PP/PP-g-MA and PP-PEI-SMA-PEI were 6.9 ± 3.3 and 7.5 ± 4.0 nm, respectively (not significantly different, $P > 0.05$). As it can be observed, pores were found on the surface of PP-PEI-SMA-PEI with a diameter of ~ 100 nm. The observed porosity may be a result of evaporation of acetone used to apply the polymers, or of water produced during condensation of maleic anhydrides with primary amines during cross-linking, as previously

278 observed [20, 53]. Thickness of the polymer coating was measured by applying a piece of kapton
279 tape to a section of the polypropylene solid support prior to coating. Kapton tape was then
280 removed to enable thickness quantification by optical profilometry. The reported coatings were ~
281 3.0 μm in thickness.

282

Figure 4. AFM images of PP/PP-g-MA (top) and as prepared PP-PEI-SMA-PEI (bottom).

Both PP/PP-g-MA and PP-PEI-SMA-PEI presented hydrophobic surfaces (θ_A values of $109.6 \pm 2.4^\circ$ and $91.8 \pm 3.8^\circ$ and θ_R values of $73.6 \pm 5.0^\circ$ and $40.2 \pm 0.8^\circ$, respectively). The retention in hydrophobicity after introducing a coating with polar antimicrobial functional groups (e.g. primary amines, *N*-halamine forming groups) was likely a result of the styrene subunits in the SMA cross-linking polymer. Static contact angles of aqueous buffered solutions (pH ranges 1-13) were acquired to determine surface pK_a values and characterize net charge of the material (Figure 5). Unlike what has been reported in prior works characterizing pK_a of interfaces containing amine groups [20, 44-46, 54], a sigmoidal trend was not observed. Indeed, θ_S values were constant across the entire range of pH values tested (Figure 5). In the cited works, an increase in θ_S has been reported in a sigmoidal fashion as pH increases, brought by the deprotonation of the amine groups, and this functional group tends to exhibit the lower part of the sigmoidal curve at low pH values [44, 45]. It is possible that the introduction of both acid and base groups (as well as nonpolar styrene units) in the reported coating introduces a chemical heterogeneity [55] that effectively neutralizes the expected positive charge at neutral pH values. Reducing the net positive charge (and therefore propensity for fouling) of an antimicrobial polymer, while retaining antimicrobial character, is significant for its commercial adoption in food and biomedical industries.

Figure 5. θ_s values for the surface pK_a determination (dotted and dashed lines are PP/PP-g-MA and PP-PEI-SMA-PEI θ_s average values, respectively, for the entire pH range tested).

3.2 Antimicrobial evaluations

The antimicrobial coating demonstrated antimicrobial efficacy in the entire range of organic matter concentration tested. At the lowest organic loads (up to 500 ppm horse serum) the chlorinated PP-PEI-SMA-PEI imparted inactivation below the limit of detection of the assay, providing > 99.99% reduction (> 4 logarithmic cycles) in *E. coli* O157:H7 (Figure 6 and Table S1). Even in its unchlorinated state, the cationic moieties present in the PP-PEI-SMA-PEI enabled between 1.9 (~ 99.0%) and 3.2 (~ 99.9%) logarithmic cycles in reduction in the presence

of up to 500 ppm horse serum. At 5,000 ppm of horse serum, chlorinated and not chlorinated PP-PEI-SMA-PEI exhibited a similar behavior, providing > 99% reduction (> 2 logarithmic cycles, ~ 99.4% in its cationic form and ~ 99.7% in its *N*-halamine form). Added bleach (positive control to mimic the amount of chlorine given by chlorinated PP-PEI-SMA-PEI) was able to inactivate *E. coli* O157:H7 under the limit of detection (1 log(CFU mL⁻¹)) from 0 to 5,000 ppm horse serum. At 50,000 ppm, in contrast to what was seen between 0 and 5,000 ppm, unchlorinated PP-PEI-SMA-PEI exhibited higher antimicrobial efficacy (90% reduction) than its chlorinated (*N*-halamine) counterpart (between 75-80% reduction). The lower efficacy as compared to the cationic form at 50,000 ppm horse serum could be explained from the necessary diffusion of chlorine to turn into hypochlorous acid (HClO) and provide an antimicrobial effect. In addition, this phenomenon can be affected by factors like pH and the presence of organic matter, which neutralizes the oxidative chlorine. Indeed, it has long been established that chlorine sanitizers perform poorly in the presence of high organic loads [13, 15, 56]. These results indicate that the mechanism of the reported coating's antimicrobial activity changes depending on the matrix in which it is applied. Under conditions of no or low organic load, chlorination of *N*-halamine groups in the coating results in enhanced antimicrobial activity, whereas at higher organic loads, the cationic moieties present in the PP-PEI-SMA-PEI dominate over *N*-halamine groups in imparting antimicrobial efficacy. Nevertheless, retention of even reduced antimicrobial efficacy of *N*-halamine derived antimicrobial materials in the presence of high organic load is significant.

An important aspect to consider for the application of this type of antimicrobial coating in the food and biomedical industries is the potential release of the antimicrobial compound, and an assessment of its potential toxicity. *N*-halamine derived polymer coatings are believed to exert

their antimicrobial efficacy either by direct oxidation of microorganisms or by dissociation from the *N*-halamine moiety as hypochlorous acid (HClO) [15]. In the current study, the maximum concentration of chlorine released from the coatings was 14.1 ± 1.3 ppm (based on the *N*-halamine quantification, which characterizes concentration of chlorine released into 1 ml test solution per cm^2 of coated PP, Figure 3), which is substantially lower than the maximum concentration (200 ppm) of NaClO (bleach) allowed for surface disinfection [47]. In addition to the lower amount necessary to impart an antimicrobial effect, no toxic effects should be expected from this type of coating. When it comes from *N*-halamines, chlorine is believed to be released and interact with its surroundings in a much slower rate than when it comes from bleach, suggesting their enhanced stability compared to bleach [13, 21]. Further, *N*-halamine polymer coatings have been suggested to be relatively safe for use in industry [16]. Nevertheless, additional studies are needed which demonstrate that the reported coatings do not pose a significant risk in terms of human or environmental toxicity.

Performance of antimicrobial materials whose mechanism is (in part) from cationic functional groups is limited if the net positive charge results in fouling of matrix components (e.g. proteins, lipids) or microorganisms. In addition to effectively blocking the antimicrobial function of the material, such fouling conditions a surface for biofilm establishment. Materials were therefore imaged by SEM after antimicrobial activity assays, under conditions of no and high organic load (0 and 50,000 ppm horse serum), with and without chlorination. The SEM images acquired after the antimicrobial evaluations confirm no evidence of bacterial adhesion (Figure S1).

Figure 6. Antimicrobial evaluation (results are average values of at least 3 replicates; statistical significance and standard deviations can be found in Table S1).

3.3 Self-healing evaluations

Figure 7 shows the IR spectra of PP-PEI-SMA-PEI after exposure to pH extremes (top: acid, bottom: alkaline) and subsequent self-healing by heating for 20 min at 140 °C. The materials exhibited excellent stability against acid degradation, as indicated by only minor changes in chemistry after exposure to 5% HCl or after subsequent self-healing. After exposure to acid, there are subtle increases in absorbances corresponding to the O-H vibration of carboxylic acids

365 and the N-H bond in-place bend of secondary amines ($3400 - 3200\text{ cm}^{-1}$ and $\sim 1550\text{ cm}^{-1}$,
366 respectively [52]), suggesting some hydrolysis of the imide ring found in the covalent bonds
367 between PEI and SMA. A decrease in the amine bend after heating at $140\text{ }^{\circ}\text{C}$ for 20 min supports
368 the reformation of the imide bond, indicating the self-healing nature of the coating. The increase
369 at $\sim 1550\text{ cm}^{-1}$ is more pronounced after exposure to 4% NaOH (Figure 7, bottom graph). This
370 band also decreases after self-healing at $140\text{ }^{\circ}\text{C}$, suggesting regeneration of the imide rings,
371 which can also be confirmed from an increase in the absorbance at $\sim 1650\text{ cm}^{-1}$, characteristic of
372 the C=O vibration of amides and imides [52]. XPS results support these observations (Figure 8).
373 Specifically, the increase in the deconvolution bands characteristic of the C–O bond from the
374 high resolution O 1s spectrum, and the decrease in the deconvolution band of O=C–N in N 1s,
375 followed by their restoration after 20 min at $140\text{ }^{\circ}\text{C}$, is suggestive of self-healing after exposure
376 to acid degradation conditions. Exposure to alkaline degradation conditions, and subsequent self-
377 healing thermal treatment, resulted in more subtle changes in the high resolution O 1s XPS
378 spectrum, but similar behavior in the high resolution N 1s spectrum as observed in acid treated
379 materials (Figure 9). As with acid treated, self-healed materials, materials exposed to alkaline
380 degradation conditions restored characteristic deconvolution bands in N 1s and O 1s spectra after
381 20 min at $140\text{ }^{\circ}\text{C}$. Full statistical analysis of XPS results is reported in Table S2. It is worth
382 noting that thermal treatment of polymeric coatings containing chlorinated *N*-halamines could
383 result in production of reactive species which may degrade the coatings. However, it is expected
384 that coatings needing regeneration would be in their unchlorinated state, either by the
385 consumption of chlorine during regular use or by incorporating a sodium thiosulfate
386 neutralization step prior to self-healing treatment.

Surfaces were further imaged by SEM to characterize morphology after exposure to acid/alkali degradation conditions and corresponding self-healing treatments (Figure S2). No obvious changes were observed and coatings retained uniformity with no evidence of cracking or delamination. Finally, no significant difference was found in the *N*-halamine content between the as prepared PP-PEI-SMA-PEI coupons ($107.9 \pm 31.2 \text{ nmol cm}^{-2}$), the self-healed after exposure to 5% HCl ($160.1 \pm 18.5 \text{ nmol cm}^{-2}$) and the self-healed after exposure to 4% NaOH ($145.0 \pm 9.1 \text{ nmol cm}^{-2}$) ($P > 0.05$). Together with spectroscopy results, these data suggest that the coatings would be robust against common cleaning and sanitization agents to which materials used in food and biomedical industries are commonly exposed, and that acid and base hydrolyzed imide bonds can be reformed by thermal treatment.

Previous works have reported that higher molecular weight polymeric coatings harboring *N*-halamines exhibit substantially more stability than small molecule *N*-halamines. However, it is not only the molecular weight but the functional groups contained in the *N*-halamine structures that dictates stability. The presence of methyl groups can bring stability to the system by providing an electron to the released chlorine in its oxidized form (Cl^+), which allows the N–Cl bond to be preserved. In contrast, the presence of carbonyl groups can have a detrimental effect on the stability of the N–Cl bond in the system as this chemical species is able to withdraw electrons, contrary to methyl groups [13]. Amide groups are prone to hydrolysis upon exposure to diluted bleach, which could result in breakdown of molecules and polymer backbones. Amine groups show more stability, and may be more resistant to hydrolysis. If these two functional groups are present within the same system harboring *N*-halamines, a substantial improvement in stability can be obtained [21, 57]. It is anticipated that the proximity of the coating to the PP/PP-g-MA support and its methyl groups could be a source of stability for the N–Cl bond. The imide

410 ring closing from the curing step is also expected to provide stability to the system and to the
411 bonds between PEI and SMA, by diminishing the presence of amide groups (prone to exhibit
412 hydrolysis) [38, 58-59].

413
414 **Figure 7.** ATR-FTIR results of the self-healing evaluations.

Figure 8. Self-healing evaluation with 5% HCl.

Figure 9. Self-healing evaluation with 4% NaOH.

4 CONCLUSIONS

The antimicrobial material PP-PEI-SMA-PEI demonstrated intrinsic antimicrobial character against *E. coli* O157:H7 both in its cationic form (giving up to ~ 99.9% reduction), and an enhanced antimicrobial effect in its *N*-halamine form (> 99.99% reduction) under increasing levels of organic matter (from 0 to 500 ppm of horse serum). At 5000 ppm of horse serum, both antimicrobial modes of PP-PEI-SMA-PEI exhibited a similar biocidal effect (between ~ 99.4% and ~ 99.7% reduction). At the maximum level of organic matter tested (50000 ppm horse serum), PP-PEI-SMA-PEI in its cationic form was more effective (~ 90% reduction) than its *N*-halamine counterpart (between ~ 75% and ~ 80% reduction), and both modes showed significant ($P < 0.05$) reduction in microbial load as compared to the controls. The antimicrobial coating was prepared through a scalable coat-cure process, resulting in a porous and hydrophobic surface. Biocidal efficacy may be further improved (reducing contact time required for inactivation and increasing log reduction values of microbial populations) by increasing the number of SMA and PEI bilayers [26, 42, 43]. The coating application process can be made more commercially translatable by use of spray deposition as has been previously reported [60, 61], as well as use of commercially available heat curing lamps. The antimicrobial coating showed self-healing properties upon heating after exposure to acid and alkali degradation conditions. Studies characterizing the stability of chlorinated *N*-halamines in the reported coatings under conditions of intended use (e.g. dry, humid, and wetted conditions) are necessary prior to commercial adoption. Indeed, stability of the N-Cl bond in *N*-halamine coatings can be a challenge to their efficacy and such instability was (in part) a motivation for integrating both *N*-halamine and cationic antimicrobial moieties into the reported coating. Further, in this work, chlorine content of the chlorinated *N*-halamines was determined by subjecting materials to a

446 rigorous rinse step in deionized water prior to chlorine quantification by DPD assay. This
447 rigorous rinse step was used to remove unbound chlorine and to allow decomposition of any less
448 stable N-Cl bonds, so that highly unstable N-halamines were not expected to be included in the
449 N-halamine quantification. Even though the stability of *N*-halamine coatings has been evaluated
450 under conditions of pH extremes, light exposure, continuous recharge of chlorine, etc.[18, 19, 21,
451 62], their performance in the presence of organic matter has not been demonstrated, probably due
452 to the well characterized neutralization of chlorine by organic matter, which limits the biocidal
453 efficacy of chlorine based sanitizers and *N*-halamine coatings alike [9]. Cationic antimicrobials
454 have been challenged against microorganisms in the presence of organic matter [31] but, in
455 contrast to *N*-halamines, their stability has not been widely evaluated [9, 20]. The reported
456 hybrid antimicrobial coatings, with both cationic and *N*-halamine moieties, self-healing
457 character, intrinsic hydrophobicity, and efficacy even under conditions of increasing organic
458 load, may support reducing microbial cross-contamination in food and biomedical industries.

ASSOCIATED CONTENT

Appendix A. A table with results from the antimicrobial evaluation, and a table with the results from the XPS analysis after the self-healing evaluations, and SEM figures after the antimicrobial and self-healing evaluations are included in Appendix A.

Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript. These authors contributed equally.

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ABBREVIATIONS

PP, Polypropylene; PP-g-MA, Polypropylene-*graft*-maleic anhydride; PEI, branched polyethyleneimine; SMA, styrene maleic anhydride copolymer; DI, deionized; HEPES, 4-(2-hydroxyethyl)-1-piperazineethane-sulfonic acid; ATR-FTIR, Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy; XPS, X-Ray Photoelectron Spectroscopy; AFM,

481 Atomic Force Microscopy; AO7, Acid Orange 7; DPD, *N,N*-diethyl-*p*-phenylenediamine; TSB,
482 Tryptic Soy Broth; TSA, Tryptic Soy Agar; SEM, Scanning Electron Microscopy; ASTM,
483 American Society for Testing and Materials.

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