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Development of Broad-Spectrum Antimicrobial Latex Paint Surfaces Employing Active Amphiphilic Compounds

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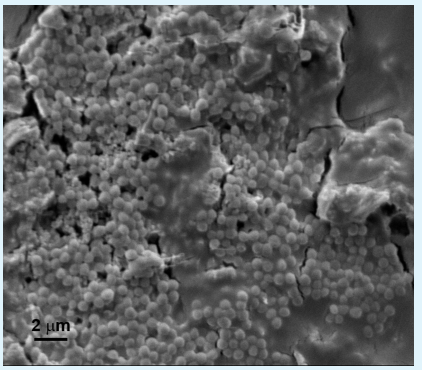
ABSTRACT: With the increase in antibiotic-resistant microbes, the production of self-decontaminating surfaces has become an area of research that has seen a surge of interest in recent years. Such surfaces, when incorporated into commercial products such as children's toys, medical devices and hospital surfaces could reduce the number of infections caused by pathogenic microorganisms. A number of active components for self-decontaminating surfaces have been investigated, including common antibiotics, metal ions, quaternary ammonium salts (QAS), and antimicrobial peptides (AMP). A recent research focus has been development of a wide range of amphiphilic antimicrobial additives that when combined with modern low volatile organic compound (VOC), water-based paints leads to a surface concentration of the active compounds as the coating cures. Herein we report the development of antimicrobial coatings containing a variety of additives, both QAS and AMP that are active against a broad-spectrum of potentially pathogenic bacteria (1−7 log kill), as well as enveloped viruses (2−7 log kill) and fungi (1−2 log kill). Additionally, these additives were compatible with water-dispersed acrylate coatings (latex paint) which have a broad range of real world applicability, and remained active for multiple challenges and when exposed to various cleaning scenarios in which they might encounter in real world situations.



KEYWORDS: self-decontaminating surface, antimicrobial, functional coating, latex coatings, quaternary ammonium salt

INTRODUCTION

With the increase in antibiotic-resistant microbes, interest in the production of self-decontaminating surfaces has become an area of research that has seen a surge of interest in recent years.[1,2] Such additives, when incorporated into commercial products such as children's toys,[3] medical devices,[4−6] and hospital surfaces,[7,8] could reduce the number of infections caused by pathogenic bacteria. Additionally, coatings presenting a surface free of microorganisms would be extremely advantageous for sanitary reasons such as in health care fields and food preparation areas, but would also have immediate use in marine environments where fouling by microorganisms increases ship drag and fuel consumption resulting in increased costs and reduced efficiency.[9,10] A number of active components for self-decontaminating surfaces have been investigated, including common antibiotics,[11,12] metals,[4−6,13] quaternary ammonium salts (QAS),[14−16] and antimicrobial peptides (AMP).[17−19]

The search for biocides with improved antimicrobial and functional performance has led to the development of several generations of cationic quaternary ammonium salts (QAS) which are widely used for the control of bacterial growth in clinical, industrial, and marine environments.[20] Quaternary ammonium salts have been used as key components in many disinfectants, fabric softeners, laundry detergents, and antistatic agents.[20,21] QAS have high biocidal activity for a wide-spectrum of biological species at minimal concentrations and can be easily tailored for desired functionality and alkyl chain length through traditional chemical synthesis.[15,16,22,23] The proposed mode of action of QAS biocides involves a strong electrostatic interaction between the positively charged QAS polymer surface and negatively charged cell wall.[24,25] Once the bacteria are adhered to the surface, the QAS's hydrophobic alkyl chain diffuses through the bacteria's cell wall and disrupts the cytoplasmic membrane, causing cell death. Additionally, an ion exchange mechanism of bacterial cell death has been proposed.[26] Alkyl chain length plays a critical role in biocidal activity of the QAS. Several studies have shown that QAS with alkyl chain lengths longer than C8 have increased biocidal activity.[27,28]

Antimicrobial peptides (AMP) are a class of short polypeptides usually associated with the host organisms innate immune system.[29] AMP have been identified in a wide range of host organisms, including plants, amphibians, fish and humans.[30−33] AMP usually consist of 30−100 amino acids and are most often cationic. In addition to a net positive charge, AMP are often α-helical and amphiphilic, containing both hydrophobic and hydrophilic domains. These properties allow for increased interaction with and insertion into negatively charged cell walls and membranes of microbes.[34] Several applications for AMP have been investigated, including therapeutic antibiotics,[29,35] medical devices,[17,18,36,37] and preservatives.[38,39] The mechanisms by which AMP exert their antimicrobial activity likely vary from peptide to peptide and are thought to be determined by such factors as structure, charge and lipid composition of the target organism.[40,41] AMP with α-helical and amphiphilic domains are

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thought to act on bacterial membranes via the 'carpet' mechanism, wherein cationic peptides coat the cell membrane via electrostatic interactions with the negatively charged phospholipid head groups present in the membrane. This leads to the formation of transient membrane pores, and eventually membrane disintegration.[42] Studies regarding the use of AMP as active ingredients in the form of surface tethered peptides,[17,43,44] as well as their use as preservatives in latex coatings as an in-can preservative,[45,46] have been reported. Additionally, the amphipathic nature of AMP makes them an excellent candidate for incorporation into self-decontaminating surfaces comprised of a hydrophilic resin. Amphipathic molecules have been shown to surface segregate within such coatings,[14] allowing for increased bioavailability of the antimicrobial component.

An exhaustive literature search revealed that reports of antimicrobial coatings involve either a single test organism,[13] or a very narrow range of organisms,[47] whereas very few investigate activity across a broad-spectrum of potentially pathogenic bacteria, viruses, and fungi.[48] Therefore, there is a clear need for the development of broad-spectrum antimicrobial coatings that are active against a broad-spectrum of potentially pathogenic bacteria, as well as viruses and fungi. Of particular interest to our laboratory are the synthesis and incorporation of novel amphiphilic biocidal additives, primarily members of the QAS and AMP families, which surface segregate and consequently allow for minimal additive. Ideally, these additives should be compatible with water-dispersed acrylate coatings (latex paint), which has a broad range of real world applicability.

EXPERIMENTAL SECTION

General. All chemicals were reagent grade and used without additional purification. Unless otherwise noted, chemicals were purchased from Sigma Inc. External elemental analyses were performed by Atlantic Microlab, Inc. Norcross, GA 30091. All melting points are uncorrected. Unless otherwise noted, ^{1}H and ^{13}C (^{1}H decoupled) NMR spectra were taken in $CDCl_3$ at 300 and 75 MHz, respectively, with a tetramethyl silane (TMS) internal standard. Chemical shifts are reported in units downfield from TMS. Coupling constants, *J*, are reported in units of Hertz (Hz).

Organisms and Media. All organisms and cell lines were obtained from the American Type Culture Collection (ATCC, Manassas, VA). *Staphylococcus aureus* (ATCC 25923) and *Escherichia coli* (ATCC 11229) were used for initial bacterial Gram-positive and Gram-negative challenges, respectively. *Pseudomonas aureginosa* (ATCC 27853), *Salmonella typhimurium* (ATCC 14028), *Klebsiella pneumoniae* (ATCC 13883), *Streptococcus pyogenes* (ATCC 19615), and *Enterobacter cloacae* (ATCC 23366) were used for bacterial challenges. *Aspergillus clavatus* (ATCC 10058) and *Stachybotrys charturum* (ATCC 201211) were used for fungal challenges. Pseudorabies Virus (ATCC VR135) and Feline Calicivirus (ATCC VR652) were used for viral challenges.

Luria−Bertani (LB) broth (Difco Laboratories, Detroit, MI) was used as a bacterial growth medium for preparation of bacteria for all bacterial challenges. Letheen broth (Difco Laboratories, Detroit, MI) was used as a dilution media postchallenge due to its ability to deactivate QASs. Malt Extract broth (Difco Laboratories, Detroit, MI) was used as a fungal growth medium for fungal challenges. All media were prepared as per the manufacturer's specifications. Pseudorabies virus was grown on VERO cells (ATCC CCL-81). Feline Calicivirus was grown on Fc3Tg cells (ATCC CCL-176). Cells were maintained in Dulbecco's modified Eagle medium (DMEM) (Invitrogen, Carlsbad, California) supplemented with 5% fetal bovine serum (FBS) and 100 μg/mL penicillin and streptomycin.

Coating. *Commercial Coating Details.* Films were prepared by the combination of 0.80 g of anionic latex paint with a solution consisting of 0.004 g of each additive dissolved in 1 mL of di-H_2O, resulting in a final loading of 1 wt % with respect to polymer solids. The final solution was allowed to stir for an additional 3 min prior to application. The Valspar interior white latex paint, anionic based paint was purchased commercially.

Control Resin. Special coatings were prepared for the XPS analysis to ensure that no ammonium or bromide contaminants were introduced as they would easily interfere with the subsequent results. The coating consisted of an anionic based proprietary blend of polyacrylates, polyvinyl chlorides and polyvinyl alcohols; however, special care was made to utilize no pigments, fillers, additives, or ionic surfactants.

Casting and Substrate. Films were solvent-cast by the delivery of a 2 mL solution via a pipet to a precleaned glass microscope slide. The glass slide was stored overnight in a sterilized, covered Petri dish to slow the rate of evaporation and generate a uniform coating. The resulting coatings were rinsed with 5 mL of di-H_2O to remove any nonincluded additive prior to subsequent examination and microbial evaluation. Larger films were prepared from the latex polymer solutions by drawing onto substrates using a #52 draw-down bar wet-film applicator. Substrates consisted of pretreated aluminum Q-panels and glass microscope slides. Aluminum Q-panel test panels and the wet-film applicator were purchased from Paul N. Gardner Company, Inc. (Pompano Beach, FL).

QAS and AMP. General Procedure for Preparation of QAS: In a 20-mL round bottomed flask equipped with reflux condenser and a positive flow of nitrogen were placed a dimethylalkyl amine (tertiary amine) (6.36 mmol), a bromo-ethyleneglycol monomethyl ether (6.36 mmol), and absolute ethanol (0.806 g, 17.49 mmol, 0.51 mL). The solution was heated in an 83 °C oil bath for 24 h. The resulting solution was allowed to slowly cool to rt and was concentrated under reduced pressure. The resulting thick yellow oil was washed with petroleum ether (2 × 3 mL), and volatiles were removed in vacuo. The resulting product was recyrstallized from 2 mL of refluxing acetone to afford the desired QAS product as a white powder.

(2-Methoxyethyl) dimethyloctyl ammonium bromide (C8EO1) (amine 1.00 g, glycol 0.88 g): FTIR: 3005, 2949, 2921, 2850, 2810, 1467, 1122, 1031 cm^{-1}. ^{1}H NMR ($CDCl_3$-δ): 3.94 (d, J = 5, 2H), 3.87 (d, J = 5, 2H), 3.87−3.62 (m, 2H), 3.43 (s, 6H), 3.39 (s, 3H), 1.34−1.24 (m, 10H), 1.78−1.72 (m, 2H), 0.88 (t, J = 7, 3H). ^{13}C NMR ($CDCl_3$-δ): 66.4, 65.9, 63.0, 59.1, 51.8, 31.6, 29.0, 28.9, 26.2, 22.8, 22.5, 14.0. Anal. Calcd for $C_{13}H_{30}BrNO$: C, 52.70; H, 10.21; N, 4.73. Found: C, 52.32; H, 10.14; N, 4.77.

Hexadecyl (2-methoxyethoxy) dimethyl ammonium bromide (C16-EO1) (amine 1.71 g + glycol 0.88 g). FTIR: 3007, 2920, 2848, 2364, 2340, 1473, 1506, 1473, 1120, 1030, 967 cm^{-1}.^{1}H NMR ($CDCl_3$-δ): 3.97 (d, J = 5, 2H), 3.88 (d, J = 5, 2H), 3.62−3.53 (m, 2H), 3.44 (s, 6H), 3.39 (s, 3H), 1.34−1.25 (m, 26H), 1.76−1.73 (m, 2H), 0.88 (t, J = 7, 3H). ^{13}C NMR ($CDCl_3$-δ): 66.4, 65.7, 59.1, 51.8, 31.9, 29.7, 29.6 (overlapping peak), 29.5, 29.4 (overlapping peak), 29.3 (overlapping peak), 29.2, 21.0, 26.1, 22.8, 22.6, 14.0. Anal. Calcd for $C_{21}H_{46}BrNO$: C, 61.74; H, 11.35; N, 3.43. Found: C, 61.92; H, 11.17; N, 3.69.

Hexadecyl [2-(2-methoxyethoxy)ethyl] dimethyl ammonium bromide (C16EO2) (amine 1.71 g, glycol 1.16 g). FTIR: 3006, 2934, 2919, 1473, 1155, 1126, 1033 cm^{-1}. ^{1}H NMR ($CDCl_3$-δ): 3.98 (d, J = 5, 2H), 3.86 (d, J = 5, 2H), 3.61−3.52 (m, 2H), 3.54 (d, J = 5, 2H), 3.52 (d, J = 5, 2H)3.44 (s, 6H), 3.40 (s, 3H), 1.33−1.26 (m, 26H), 1.77−1.74 (m, 2H), 0.87 (t, J = 7, 3H). ^{13}C NMR ($CDCl_3$-δ): 73.1, 70.1, 66.4, 65.8, 59.0, 51.5, 31.8, 29.7, 29.6 (overlapping peak), 29.6, 29.5 (overlapping peak), 29.4 (overlapping peak), 29.3, 20.9, 26.0, 22.7, 22.6, 14.1. Anal. Calcd for $C_{23}H_{50}BrNO_2$: C, 61.04; H, 11.14; N, 3.10. Found: C, 61.28; H, 10.83; N, 2.97.

Peptides Chr-1 and Chr-3 were obtained from Biosynthesis, Inc. (Lewisville, TX). Peptides were synthesized then purified by HPLC.

Peptides arrived lyophilized, and were resuspended in sterile H_2O. For surface challenges, this peptide mixture was added to the aforementioned commercial acrylate coating resin system at 1 wt % solids.

Microbial Challenge. *Bacterial Challenge.* Bacteria were grown at 37 °C. Log phase cells were harvested by centrifugation, counted on a hemocytometer using bright field microscopy, pelleted by centrifugation at 4000 xg for 10 min, and resuspended in PBS at a concentration of 1×10^9 CFU/mL. To prevent desiccation of the bacteria during testing, was prepared a hydration chamber. The chamber consisted of a sterile 3 × 3 in. gauze pad placed in the bottom of a sterile 150 × 15 mm Petri dish. The gauze pad was saturated with 5 mL of sterile water and the test samples placed on top. A 10 μL aliquot containing 1×10^7 bacteria was added to each test coating (280 mm^2), and then placed in a hydration chamber at room temperature. After 2 h of incubation, the remaining bacteria were recovered by placing the coating in a tube containing 5 mL sterile Letheen media, followed by 30 s of vortexing. Serial dilutions were carried out, and incubated for 18 h at 37 °C with agitation. Following incubation, the cultures were examined for the presence of turbidity, indicating bacterial growth. Each coating was tested in triplicate. Log kill was determined by the following: Log kill =7 − highest dilution exhibiting bacterial growth. All bacterial challenge procedures were conducted using standard aseptic techniques in a BSL-2 hood.

Fungal Challenge. Fungi were grown at 28 °C. Spores were harvested from 72 h cultures, counted, and adjusted to 1×10^7 spores/mL. A 10 μL aliquot containing 1×10^5 spores was added to each test coating and placed in a hydration chamber at room temperature. After 2 h of incubation, spores were recovered as described previously, followed by serial dilution. Dilutions were incubated at 28 °C for 72 h, and examined for turbidity indicating fungal growth. Log kill was determined by the following: Log kill = 5 − highest dilution exhibiting fungal growth.

Viral Challenge. Viruses were grown on the previously mentioned permissive cell lines. Viruses stocks were generated, titerd, adjusted to 1×10^8 PFU/mL and stored at −80 °C. A 100 μL aliquot containing 1×10^7 virus particles was added to each test coating in the well of a 24-well plate. Plates were incubated at room temperature for 2 h, followed by washing with 900 μL of DMEM and a 7-fold serial dilution onto the respective cell line. Cells were then incubated at 37 °C with 5% CO_2 until the onset of visible cytopatic effect (CPE) due to virus infection. Cells were washed 3× with PBS, fixed with 0 °C methanol, and stained with crystal violet. Plaques were then counted, and log kill was determined to be the lowest dilution with no apparent viral plaques present.

Surface Washing. Coatings containing C16EO1 were washed with each of 7 commonly used commercial cleaners. The following liquid cleaners were used at full strength: Windex with Ammonia (SC Johnson, Racine, WI), Formula 409 (The Clorox Company, Oakland, CA), and Simple Green (Sunshine Makers, Inc., Huntington Beach, CA). Powdered and concentrated detergents were suspended in deionized water at 20% w/w: Tide and Dawn (Proctor and Gamble, Cincinnati, OH), and Borax (Dial Corporation, Scottsdale, AZ). Clorox wipes (The Clorox Company, Oakland, CA) were also employed. For all cleaners except the Clorox Wipes, coatings were submerged in the solution for five minutes, followed by a 3× rinse with sterile deionized water and subsequent overnight air drying. Clorox Wipes were used to wipe coatings 3×, followed by 5 min of direct contact between the wipe and coating. Coatings were then rinsed and air-dried as previously described. Coatings were then challenged with *S. aureus* and *E. coli* as described above.

Multiple Challenge Studies. Coatings containing C16EO1 were used in multiple challenge studies. Tests were conducted as described above with the exception that after vortexing to recover bacteria, coatings were recovered from the sample tube, rinsed with water then 70% EtOH removing any residual bacteria or media, and rechallenged. This was conducted a total of 10 times using *S. aureus*.

Leaching Studies. A glass microscope slide coated with latex resin containing ~1 wt % C16EO1 was immersed in a test tube containing 150 mL deionized water for 30 days with periodical analysis of the water. Analysis of the water was done using Spectra-Physics HPLC equipped with both refractive index and FID detectors (approximately detection limit 5−10 ppm) employing an Ultra IBD column by Restek. Concentration was determined employing a calibration curve.

Table 1. Organisms Used for Surface Challenges

organism	classification	ATCC strain number
Escherichia coli	Gram-negative	11229
Staphylococcus aureus	Gram-positive	25923
Pseudomonas aureginosa	Gram-negative	27853
Salmonella typhimurium	Gram-negative	14028
Klebsiella pneumoniae	Gram-negative	13883
Streptococcus pyogenes	Gram-positive	19615
Enterobacter cloacae	Gram-negative	23366
Aspergillus clavatus	fungus	10058
Stachybotrys charturum	fungus	201211
pseudorabies virus	envelope virus	VR135
feline calicivirus	nonenvelope virus	VR652

Surface Characterization. *XPS Analysis.* X-ray photoelectron spectroscopy (XPS) was conducted on a Physical Electronics 5400 XPS. The biocide-containing latex coatings were dried on gold surfaces for XPS analysis. Unmonochromated Mg Kα radiation, 15 kV, 400 W on a 1 nm diameter analysis area were employed and a takeoff angle of 45° was utilized. Pass energy of 178.95 eV for survey scans and 35.75 eV for high-resolutions scans were employed. Calculation of expected bulk concentrations of biocide nitrogen in the materials was done by calculating the weight % of N in the biocide molecule, excluding hydrogen, and multiplying it by 0.01 (biocide was loaded into a specially prepared control coating at 1 wt.%). Values for bromine were calculated similarly, and compared accordingly. Although the bromine results were generally in close agreement with the nitrogen results, they were both considered because of the possibility of ion exchange that may have resulted from impurities. The calculated weight percent exclude hydrogen so as to get an accurate comparison with XPS results. XPS does not detect hydrogen, so all of the measured atomic and weight percent in XPS exclude hydrogen.

RESULTS AND DISCUSSION

Organisms and Biocides. Often, antimicrobial coatings of the type described herein are suggested as methods to reduce the spread of pathogenic microbes. However, rarely are these coatings tested against a broad-spectrum of potential pathogens, including bacteria, viruses, and fungi. To fully characterize the broad-spectrum capabilities of coatings containing amphiphilic biocides, we employed a wide range of test organisms, which are summarized in Table 1.

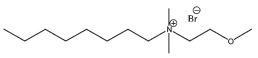
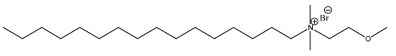
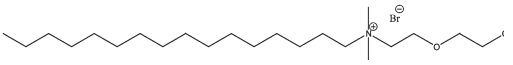
Previously, our laboratory has reported on the development of coatings containing amphiphilic biocides with the ability to self-concentrate at the air-coating interface.[14,19] Use of additives such as these with both hydrophilic and hydrophobic domains allow for interactions between hydrophilic acrylate coatings, which result in the hydrophobic regions being self-orientating and self-segregated to the coating-air interface (surface) upon coalescence/subsequent solvent evaporation. Because of this unique ability, biocide additives can be employed at lower concentrations than would otherwise be possible, while at the same time retaining both antimicrobial effectiveness and, equally important, the pertinent physical properties of the coating. In a potential

commercial product, it is essential to afford a final product that will maintain properties such as: uniform color, fast drying, low-temperature application, adhesion to a variety of substrates and washability. To ascertain the effect that modifying the hydrophobic/hydrophilic balance has on biocidal effectiveness, we employed select highly active biocidal moieties (Table 2).

Surface Challenges. Previous work in our lab has shown that amphiphilic compounds, when incorporated into a waterborne, low volatile organic compound (VOC) paint, exhibit significant antimicrobial activity against test organisms[14,19] as compared to nonamphiphilic compounds. All QAS and one AMP (Chr-1) exhibited high biocidal activity against both Gram-positive and Gram-negative challenges. Additionally, it should be noted that in every case the additives were more effective against *S. aureus* (Gram-positive) than *E. coli* (Gram-negative). Increasing hydrophobic portion of the biocide moiety, either by altering the alkane chain length for QAS (C8 vs C16) or hydrophobic domain size in AMP (more hydrophobic amino acids) increased activity. However, altering hydrophilic portions of biocides had less effect on biocidal activity. This can most likely be attributed to availability at the surface of the coating as well as differences in interactions between the different cell wall composition of the bacteria (Gram-positive vs Gram-negative) and the coatings. Gram-negative bacterial cell walls comprised an additional membrane, comprising phospholipids and lipopolysaccharides, outside of the peptidoglycan layer, which appears to confer a measure of additional protection.

To further assess the ability of coatings to self-decontaminate, we employed a broader spectrum of potential pathogens. As such, coatings were challenged with an additional five strains of bacteria, in addition to two fungi and two viruses, with results shown in Figure 1. Surface testing demonstrated varying biocidal activity of the five tested biocides against all bacterial strains screened with *E. cloacae* and *S. pyogenes* (7 log kill) showing the most susceptibility, whereas none of the tested biocdes were active against *S. typhimurium*. This is not unexpected, as previous reports of many QAS compounds have indicated very low or no activity against *Salmonella*.[49] This is likely due to the structure of the QAS, as QAS with multiple alkyl chains have proven effective, whereas single alkyl chains, as present in C8EO1, C16EO1, and C16EO2, have proven less effective. Consistent with previous

Table 2. Structures of Active QAS and Sequences of AMP Employed

Biocide	Structure/Sequence [a]
C8EO1	
C16EO1	
C16EO2	
Chr-1	FFWLIKGAIHAG*KAIHGLIHRRRH*
Chr-3	FIGLLISAG*KAIHDLIRRRH*

[a] Hydrophilic domain of AMP is in italics.

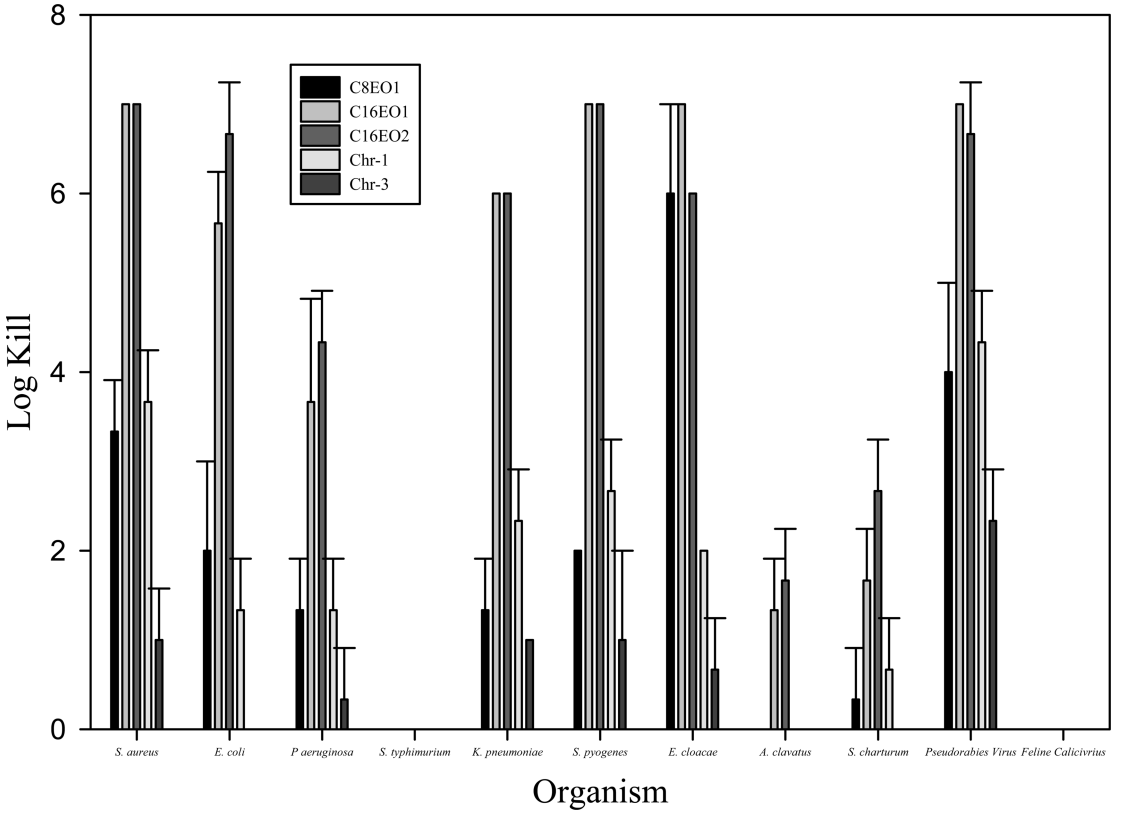


Figure 1. Antimicrobial results for acrylate coating at 1 wt % biocide loading. Control coatings containing no biocide were also tested, exhibiting no activity (data not shown).

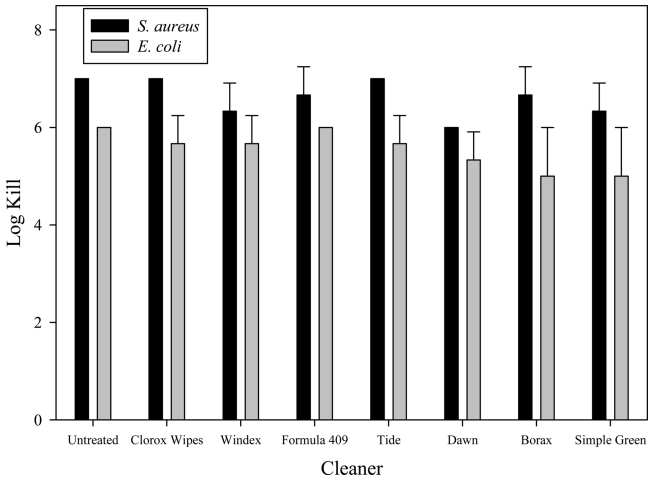


Figure 2. Log kill of acrylate coating containing 1 wt % C16EO1 additive after exposure to common commercial cleaners.

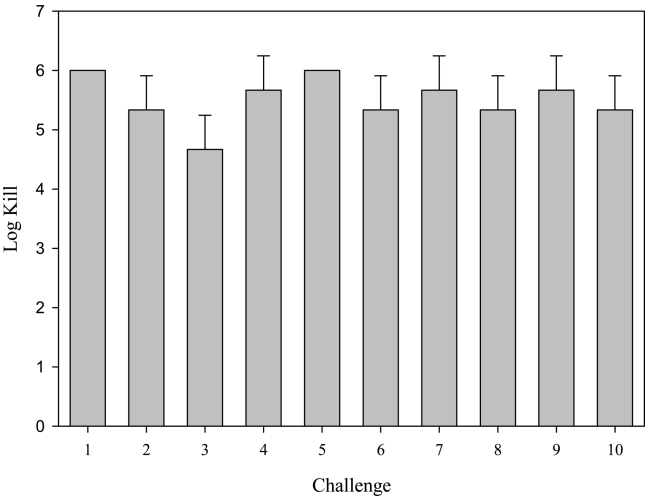


Figure 3. Log kill of coating containing 1 wt % C16EO1 against *S. aureus* upon multiple challenges as compared to control coating containing no biocide, which exhibited 0 log kill for all challenges (data not shown).

results obtained, C8EO1 generally showed reduced activity (1−6 log kill) against bacteria than did the QAS with longer alkyl chains (4−7 log kill). Across the board, AMPs exhibited significantly lower activity than QAS. The exact reason for this is not well understood, but may be due to increased interactions between the anionic resin and highly cationic peptides. This is believed to result in a lessening of both surface concentration and interactions between AMP and microbes. C16EO1 and C16EO2 were effective against the fungal spores of *A. clavatus* and *S. chartarum* (1−3 log kill), while C8EO1 and the AMPs showed almost no activity against fungal spores. All compounds tested showed high (2−7 log kill) activity against Pseudorabies virus, but were ineffective against Feline calicivirus. This finding is consistent when considering the mode of action of these biocides and the structure of the two viruses. Pseudorabies virus is an enveloped virus, possessing a lipid membrane acquired from its host cell. In addition, this membrane contains receptor proteins necessary for the virus to bind and infect cells. These biocides work by disrupting lipid membranes of bacteria, and similarly can disrupt the viral envelope of pseudorabies virus. Feline calicivirus is a nonenveloped virus, and as such, has no lipid membrane to be targeted by these biocides.

Effect of Real World Situations on Coating Effectiveness. *Exposure to Common Wash Solutions.* To further evaluate the durability of coatings with incorporated biocide in real World situations, we undertook a series of experiments to expose coatings to washes involving common household cleaners, as well as to expose a single coating to multiple bacterial challenges. C16EO1 was chosen as the test biocide due to its superior activity against a wide range of potential pathogens. Coatings were washed with the indicated wash solution, rinsed thoroughly, and subjected to both G-positive (*S. aureus*) and G-negative (*E. coli*) challenges (Figure 2). In general, washing with common cleaners had very little effect on the antimicrobial activity of coatings, with only the coatings washed in Dawn exhibiting a very low (1 log) reduction in biocidal activity.

This is possibly due to anion exchange with the chloride anion (Cl^-) which has been reported to slightly diminish activity.[50]

Multiple Challenges. To assess the durability of the antimicrobial coatings, we exposed a single coating to multiple challenges of *S. aureus* (Figure 3). Coatings were exposed to bacteria as before, and were washed and sterilized after bacterial recovery, yielding clean coatings for the subsequent challenge. This process was repeated 9 times, yielding a 10× challenge study. Coatings containing the QAS C16EO1 demonstrated no reduced ability to decontaminate a challenge with 10^7 CFU of *S. aureus* after 10 consecutive challenges, indicating stability of the biocide within the coating and ability to function effectively repeatedly.

Table 3. Thirty-Day Leach Study of Coating Containing C16EO1 in Water

time (h)	concentration of leachate (mM)
0	0.00
24	0.00
48	0.00
120	0.00
240	0.00
360	0.002
720	0.014

Leaching Studies. To ascertain the degree of leaching of the QAS into solution of a coating submerged in water for prolonged periods, an experiment was conducted and result tabulated in Table 3. Analysis of the water was performed by HPLC to determine the concentration of the leachate in the water. No detectable quantity was observed until day 15, in which 0.002 mM was calculated. This dramatically increased to 0.014 mM on the conclusion of this study at day 30. Despite leaching a significant quantity, it is viewed that a real world latex coating would not be totally submerged for such an extended period and thus would not serve as an environmental concern. In addition to analysis of the leachate, the postleached coating was found to provide kill levels identical to a freshly prepared surface.

Surface Characterization. XPS analysis was used to further characterize the surface as well as attempt to correlate the antimicrobial activity with proposed surface concentration. As previously described, a control resin was used in attempt to provide a coating that would be free of ionic surfactants and

Table 4. XPS Data from QAS-Containing Coatings

	C% XPS	O% XPS	N% XPS	N% expected	Br% XPS	Br% expected
control	63.3	27.7	0.0	0.0	0.0	0.0
C8EO1	63.3	26.4	0.4	0.08	0.4	0.19
C16EO1	59.3	26.3	0.5	0.05	3.0	0.3
C16EO2	59.8	25.2	0.9	0.05	3.4	0.26

halide salts which would interfere with the results. In the control resins, the results are as expected with no observation of quaternary nitrogen or bromine. The addition of the QAS had a dramatic effect, as the surface enhancement was obvious. Because the XPS analyses provided two handles for monitoring surface segregation, we elected to utilize the Br because the results are more modest and actually correlate more closely with antimicrobial activity observed. We do realize that the agreement in all cases is not desirable; however, this same discrepancy was observed in multiple analyses. Although it is only a guess at best, this could be due to the overlap of the quaternary nitrogen peak. The C8EO1 exhibited a 2-fold enhancement, whereas the C16EO1 and C16EO2 exhibited a 10- and 13-fold enhancement, respectively. These data (Table 4) correlate very closely with the observed surface antimicrobial data presented in Figure 1, and indeed confirm that there is a surface enrichment effect as predicted.

Bromophenol blue has been used extensively to quantify the amount of protein or peptide in a solution.[51,52] A test method to visualize surface peptide using bromophenol blue was adapted from these solution test methods and was employed as previously noted.[19] Approximately 4.5% of Chr-1 and 1% of Chr-3 was present at the coating surface. This correlated directly to the observed activity of the coatings containing peptides knowing that the coatings were prepared at a 1 wt % loading.

CONCLUSION

In conclusion, we report the development of antimicrobial latex coatings containing a variety of additives, both QAS and AMP, that are active against a broad-spectrum of potentially pathogenic bacteria (1−7 log kill), as well as enveloped viruses (2−7 log kill) and fungi (1−2 log kill). However, coatings were ineffective against challenges with *S. typhimurium* as well as Feline Calicivirus, a nonenveloped virus. Additionally, these additives were compatible with water-dispersed acrylate coatings (latex paint) which have a broad range of real world applicability, and remained active for multiple challenges and when exposed to various cleaning scenarios in which they might encounter in real world situations did not significantly diminish activity. Additionally XPS data confirm a 10-fold surface enhancement and data correlate with observed broad-spectrum antimicrobial activity.

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