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Cite this: DOI: 10.1039/c0xx00000x

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ARTICLE TYPE

Durable defense: robust and varied attachment of non-leaching poly“-onium” bactericidal coatings to reactive and inert surfaces

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5 Received (in XXX, XXX) Xth XXXXXXXXX 20XX, Accepted Xth XXXXXXXXX 20XX

DOI: 10.1039/b000000x

Developing antimicrobial coatings to eliminate biotic contamination is a critical need for all surfaces, including medical, industrial, and domestic materials. The wide variety of materials used in these fields, from natural polymers to metals, require coatings that not only are antimicrobial, but also contain
10 different surface chemistries for covalent immobilization. Alkyl “-onium” salts are potent biocides that have defied bacterial resistance mechanisms when confined to an interface. In this feature article, we highlight the various methods used to covalently immobilize bactericidal polymers to different surfaces and further examine the mechanistic aspects of biocidal action with these surface bound poly“-onium” salts.

15 Surface contamination by microbes is a universal challenge in medical,¹ domestic,² and industrial settings.³ For example, contact contamination of methicillin resistant *Staphylococcus aureus* (MRSA) is a crisis in medical settings, tripling the stay time and costs for affected individuals, and quintupling the
20 likelihood of death as of 2001.⁴ Food product contamination by MRSA is common, with one 2010 study finding that 24% of U.S. poultry and meat contains MRSA⁵. Because of these and many other examples, materials science and engineering has begun to address surface contamination by attempting to imbue or coat
25 various substrates with chemistries that are antimicrobial and are self-sterilizing.

In terms of coating technologies, several different chemistries have been applied to address this problem, the most common of which release an antimicrobial agent such as triclosan,⁶ chlorine
30 from N-halamines,⁷ silver ions,⁸ or conventional pharmaceutical antibiotics⁹ slowly over time. While effective, these “leaching” strategies are hampered by depletion of the antimicrobial functionality from the coating unless they are actively replenished or “recharged.” These materials also have a tendency to
35 contaminate their surroundings. A prime example of contamination by leaching triclosan, which has been found in significant concentrations in U.S. wastewater.¹⁰ Alongside these antimicrobial agents are membrane-disrupting “-onium” cations. The most extensively investigated “-onium” salts are alkyl
40 ammonium and alkyl pyridinium compounds, whose small molecule versions are found in commercial disinfectants such as Lysol™. One advantage of these materials is that “-onium” salts are biocidal even when immobilized to different surfaces. This non-leaching behavior circumvents environmental contamination,
45 and also minimizes the risk of organisms developing resistance to the “-onium” salt’s mechanism of biocidal action.

Bacterial membrane physiology

The biocidal activity of surface bound “-onium” salts is based on their ability to disrupt the bacterial cell membrane. Therefore, it is
50 important to describe the physiology and chemical makeup of the membrane to better understand the mechanism of action. The bacterial cell membrane of a Gram-positive bacterium is primarily composed of two layers: the peptidoglycan layer and the lipid bilayer. The peptidoglycan layer consists of alternating
55 N-acetylglucosamine and N-acetylmuramic acid chains, with a tetrapeptide extending off the N-acetylmuramic acids.¹¹ The peptidoglycan layer is cross-linked via peptide interbridges between the tetrapeptides that form a protective barrier around the bacterium. The next layer is the lipid bilayer (LB), which consists
60 of outwardly facing polar groups and inwardly facing long hydrocarbon chains. These phospholipids contain headgroups that are either zwitterionic, such as phosphatidylethanolamine and phosphatidylcholine, or negatively charged, as is the case with phosphatidylglycerol and phosphatidylserine. While the specific
65 phospholipid content varies among organisms, and even changes with metabolic cycle, a bacterial cell membrane typically has a net negative charge. The membrane of a Gram-negative bacterium is more complex, consisting of an outer leaflet with lipopolysaccharides (LPS) that are anchored to the outer lipid
70 membrane by the LPS lipid A domain,¹² and an inner membrane consisting of phospholipids. Between these two lipid bilayers is the periplasm, an area of low density that contains a peptidoglycan layer similar in structure to that of Gram-positive bacteria, but usually much thinner.¹³

75 Mechanism of bactericidal action with leaching “-onium” salts

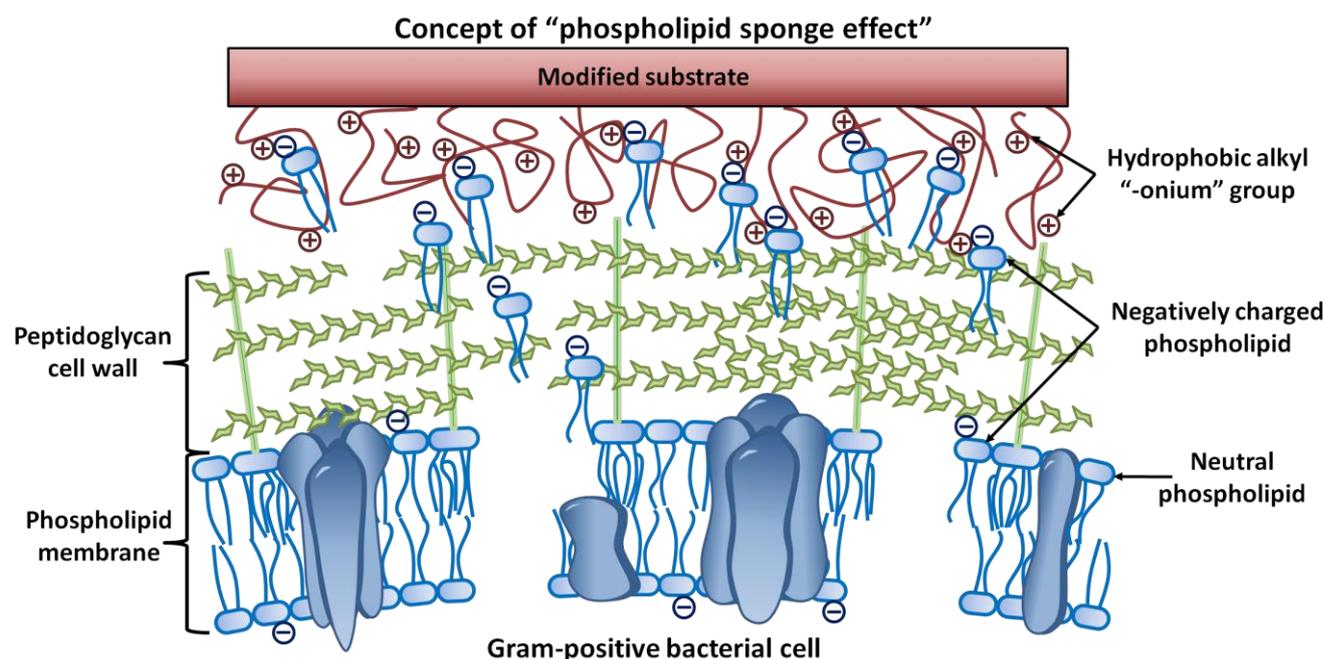


Fig 1 Schematic of the "phospholipid sponge" effect. Anionic phospholipids are being absorbed from the cell membrane and sequestered into the polymer matrix.

There are two main hypothesized mechanisms of action involving leaching alkyl "-onium" biocidal activity. Both rely on the electrostatic attraction between the net-negatively charged lipid bilayer surface and the positive charge of the "-onium" cations.

First, the alkyl "-onium" functionality must permeate through the peptidoglycan layer of Gram-positive bacteria, or through the LPS and peptidoglycan of Gram-negative bacteria. The first mechanism suggests an electrostatic interaction in which the alkyl "-onium" cations displace the divalent cations of the LB (primarily Mg^{2+} and Ca^{2+}) which serve as counterions to the anionic phosphate groups.¹⁴ This displacement damages the structural integrity and organization of the surface of the lipid bilayer, which increases its permeability and facilitates leaking of internal cellular content out of the cell.

The second proposed mechanism of biocidal action occurs when the hydrophobic alkyl functionalities are brought into close proximity to the membrane. These hydrophobic tail-groups intercalate into the phospholipid hydrocarbon chains and disrupt bilayer organization, which has the consequence of creating holes in the membrane.¹⁵ Work by Klivanov demonstrated that some bacteria, such as *P. aeruginosa*, that are immune to quaternary amines (QA) in solution,¹⁶ are still susceptible to similar functionalities that are tethered to a surface.¹⁷ Klivanov also demonstrated that *S. aureus* and *E. coli* do not develop resistance to surface bound PQAs (polymer quaternary ammoniums).¹⁸ Based on this evidence, it is possible that the biocidal mechanism of action in surface bound, non-leaching "-onium" cations may deviate from that of free, solvated, "-onium" salts.

Mechanism of bactericidal action with non-leaching "-onium" salts

The Busscher group has recently demonstrated proof of a third mechanism of biocidal action that applies only to surface bound

cationic antimicrobials.¹⁹ This study, which uses hyperbranched surface bound quaternary amine polymers, demonstrated powerful adhesive forces (~100 nN via bacterial probe atomic force microscopy) between the negatively charged bacterial cell membrane and the positively charged polymers. These electrostatic-based adhesive forces, which are orders of magnitude higher than those ordinarily experienced by a bacterium, disrupt the bacterium's ability to grow and reproduce.

A related alternative to the electrostatic adhesive force hypothesis was put forward by Tiller, the "phospholipid sponge effect," which hypothesizes that the poly"-onium" films pull anionic phospholipids directly out of the bacterial membrane and sequesters them within the polymer matrix, causing the observed holes in the cellular membrane (Fig 1).²⁰ This hypothesis is also supported by research done by Li et al,²¹ whose work offers a similar conclusion of "suctioning" off anionic phospholipids from bacteria and sequestering them within cationic hydrogels.

It is still too early to definitively identify one mechanism as the primary biocidal route of surface tethered "-onium" cations, although there appear to be differences between the mechanism of action of solutions containing poly"-onium" salts and cations that are confined to a surface. However, the ultimate result involving any of these mechanisms is formation of holes in the cell membrane, which has been demonstrated by several groups, and is illustrated by the excellent scanning electron microscope (SEM) images published by the Li group (Fig 2).²¹ Remarkably, there is also evidence that fungi and viruses are also vulnerable to surface bound "-onium" cations,²² although the pathways to biocidal action with these classes of materials are even less well understood.

Attachment chemistry

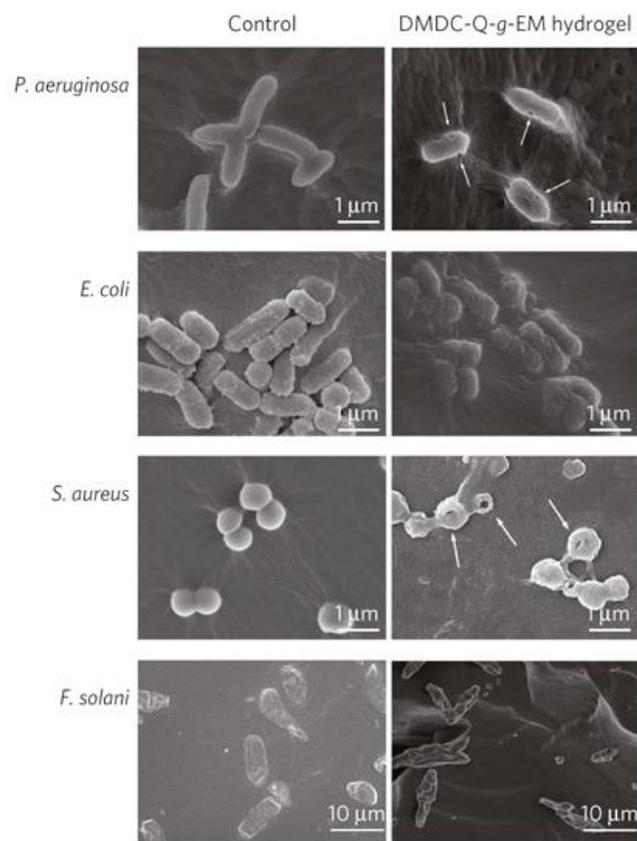


Fig 2 SEM of bacteria on a control sample (left) and on a quaternary amine hydrogel (right). Arrows indicate hole formation in cellular membrane. There is also notable deformation of the cell membrane of each bacterium on the hydrogel surface. Reprinted by permission from Macmillan Publishers Ltd: Nature Materials (P. Li, Y. F. Poon, W. Li, H.-Y. Zhu, S. H. Yeap, Y. Cao, X. Qi, C. Zhou, M. Lamrani, R. W. Beuerman, E.-T. Kang, Y. Mu, C. M. Li, M. W. Chang, S. S. Jan Leong and M. B. Chan-Park, *Nat. Mater.*, 2011, **10**, 149. Copyright 2011.

There are numerous methods described in the literature that are used to coat surfaces with antimicrobial materials. In some cases the affinity of an antimicrobial compound to the surface (physisorption, either through electrostatic or hydrophobic forces) can be strong enough to resist leaching, such as in Klivanov's pioneering work in which quaternized polyethyleneimines were painted onto glass,²³ which was one of the most critical developments for the field. However, many applications require more robust interactions, where it is necessary to use covalent attachment strategies (chemisorption) to ensure that the antimicrobial agent does not leach and remains confined to the surface. Depending on the nature of the functional groups available and the type of material, a wide variety of attachment chemistries have been employed. The most common is silane chemistry, which can react with -OH groups found on cellulose,²⁴ silicon oxide,²⁵ and certain metal oxides.²⁶ Hydroxyl and carboxylate attachment points can also be generated on inert materials by exposure to plasma²⁷ or chemical oxidants.²⁸ Broader nucleophilic substitution reactions, such as between an amine and a surface bound alkyl or acyl halides,^{22d} have also been employed as a covalent grafting strategy. The chemical functionality employed in dyeing textiles, which occur mostly through condensation reactions with cellulose, can also be used to

attach antimicrobials.²⁹ Finally, more exotic attachment chemistries which employ reactions that form C-C or N-C bonds with inert surfaces,³⁰ or take advantage of powerful dopamine adhesion chemistry,³¹ have been used to tether antimicrobials to surfaces. Recently, several review articles have highlighted surface antimicrobial “-onium salts”.^{15, 32} In this feature article, we specifically focus on the robust attachment of non-leaching poly“-onium” salt-based antimicrobial compounds to surfaces, and pay special attention to non-traditional methods of attachment. We have chosen to exclude the attachment of “-onium” salts to nanoparticle surfaces,^{22c, 33} which are usually not themselves tethered to bulk surfaces, and therefore can leach into solution. The rest of this article is organized according to the general classes of surfaces and materials that have been coated: natural polymers, synthetic polymers, and inorganic surfaces such as metals and oxides.

40 Polymer surfaces

Natural textiles

The derivatization of natural textiles, primarily cellulose, with antimicrobial functionality is an enormous frontier for both medical and commercial applications. Antimicrobial wound care technologies represented 11% of the \$11.7 billion dollar global wound care market in 2013.³⁴ Commercial products which boast “anti-odor” properties due to antimicrobial agents engineered into fabrics are abundant, with a prominent example being triclosan-containing Microban™ coatings. The overwhelming majority of these coatings rely heavily on compounds that work via leaching mechanisms that are detrimental for the reasons described above, and therefore work on non-leaching antimicrobial textiles surfaces via poly“-onium” salts holds great promise.

One of the first reports using conventional cross-linkers to fix poly“-onium” cations to cellulose was described by Kim et al.,³⁵ who employed a chitosan derivative, *N*-(2-hydroxy)propyl-3-trimethylammonium chitosan chloride (HTCC), as an antimicrobial functionality to derivatize cotton. The authors employed dimethyloldihydroxyethylene urea (DMDHEU), butanetetra-carboxylic acid, and citric acid as a strategy to cross-link HTCC to cellulose through condensation using the -OH groups found on both HTCC and cellulose. While DMDHEU was ineffective, both polycarboxylic acids were successful in functionalizing cotton with HTCC. A 0.1% weight loading of cotton was found to be sufficient to reduce the population of *S. aureus* by $\geq 91\%$ even after 20 laundering cycles.

More precise methods of imbuing textiles with biocidal properties (which are likely also less scalable) have utilized “grafting from” polymerization techniques. Lee and Matyjaszewski demonstrated the functionalization of paper with a PQA derived from the post-polymerization quaternization of poly(2-(dimethylamino)ethyl) methacrylate (PDAEMA) using ethyl bromide.³⁶ The initiator, 2-bromoisobutyl bromide, was covalently attached to the surface of filter paper via condensation with the -OH groups. The PQA was then grown from the surface via surface initiated atom transfer radical polymerization (SI-ATRP). A ~ 6 cm² piece of paper functionalized with these coatings was able to kill up to 10^9 bacteria in minutes. The coatings were ineffective after washing, since the substrate began to fragment and degrade under the stresses of washing. For this reason the authors used a more robust substrate, silicon wafers, to investigate the role of washing on the PQA. On SiO₂, it was noted that after exposure to bacteria, substrates washed with pure water became deactivated after two

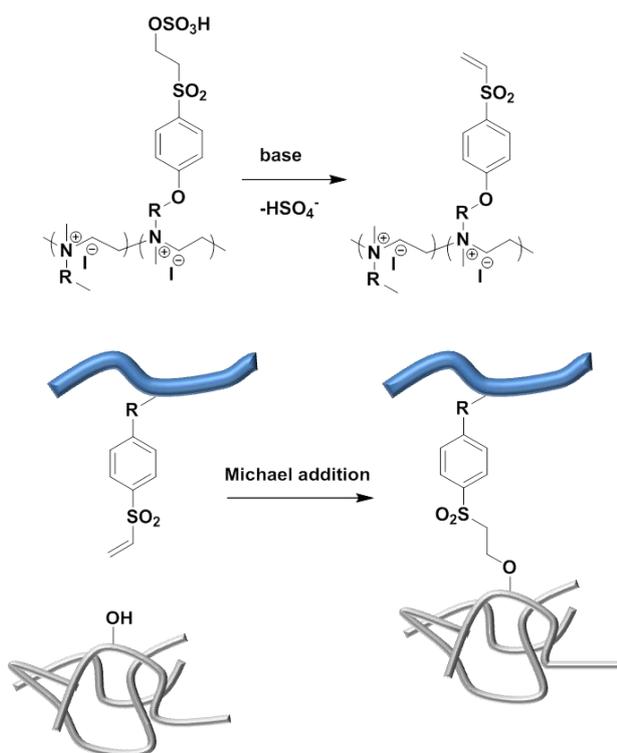


Fig 3 Creation of the reactive vinyl sulfone by exposure of pendant sulfate group to basic conditions, followed by attachment of the antimicrobial polymer to cellulose via Michael addition.

bacterial challenge/wash cycles. However, surfaces washed with a sodium dodecylsulfate detergent retained their efficacy. Furthermore, washing with detergent could be used to reactivate surfaces that had been rinsed with pure water and were deactivated through exposure to and killing of bacteria. The authors hypothesized that dead bacteria clogged the surface, and were responsible for deactivation. These bacterial carcasses could not be removed by rinsing with pure water. Detergents, however, were capable of dislodging the dead cells, which re-exposed the coating to the environment, and renewed biocidal activity.

Roy et al used a similar approach, and polymerized DAEMA via reversible addition-fragmentation chain transfer (RAFT).³⁷ The PDAEMA was then quaternized by post-polymerization modification with alkyl chains ranging from C₈ to C₁₆ in order to study the effects of substituent length on the biocidal efficacy of quaternized PDAEMA. The authors observed a trend of decreasing antimicrobial efficacy as the chain length of the alkyl group was increased from C₈ to C₁₆. Maximum efficacy was observed with the C₈ polymer, which reduced an initial concentration from 10⁶ colony forming units (CFU) of *E. coli* per mL to <100 CFU/mL.

“Grafting to” approaches are another popular method of functionalizing fabrics, and the most relevant for industrial application. Our lab recently employed a polymer that used a tethering functionality that requires no pretreatment or separate crosslinkers to “graft to” cellulose. We synthesized a polyethyleneimine (PEI) polymer quaternized with C₁₂ chains and pendant phenylsulfonylethyl sulfate groups (Fig 3).²⁹ The sulfate group undergoes elimination in the presence of base which produces a vinyl sulfone, that is susceptible to attack via Michael addition with soft nucleophiles,³⁸ like cellulose’s pendant –OHs. The polymer was tethered to cellulose after exhaustion onto fabric at 45 °C from aqueous solution at pH 9. The resulting coating was shown to be effective against *E. coli* and *S. aureus*.

These previous results are interesting, and initiate discussion on the wider topic of alkyl chain length and its influence on biocidal activity involving alkyl “-onium” cations. The study by Roy makes a strong case that C₈ is the appropriate alkyl chain length necessary to maintain the hydrophobic/hydrophilic balance required for antimicrobial activity in surface confined polymers. They cite several studies, which observe this peak in efficacy with C₈ alkyl chains.³⁹ However, we argue that this assumption can lead to oversimplifications, and the resulting pendant group size strongly depends on the nature of the polymer backbone. For example, Klibanov found that with surface bound alkylated poly-4-vinylpyridine (P4VP) coatings, C₆ chains had the highest biocidal activity, with C₈ alkyl chains displaying ~8x less antimicrobial efficacy.¹⁷ In the case of alkylated PEI derivatives, very long alkyl chains (C₁₂ and C₁₈) displayed the highest biocidal activity.^{33b} Specific we argue that alkyl chain length is not the primary factor that governs biocidal efficacy, but rather it is the net sum of all the hydrophobic/hydrophilic interactions in the polymer (backbone and side-chain) that determine the outcome. Each of these studies used different polymer backbones, ranging from hydrophobic (P4VP) to hydrophilic (PEI). As polymer architectures increase in complexity through eloquent design, predetermining factors such as pendant side-chain length becomes more difficult and can lead to materials with less than optimal efficacy.

Another critical factor in designing antimicrobial polymers is molecular weight. It has been demonstrated by Klibanov using quaternized polyethyleneimine (Q-PEI) that molecular weight plays a critical role in determining biocidal efficacy of surface coatings.²³ This work demonstrated that a 750 kDa, Q-PEI killed effectively all of the bacteria sprayed as an aerosol onto the surface, with the biocidal efficacy decreasing when lower molecular weight Q-PEIs were used. As an example, a 2 kDa Q-PEI was shown to kill less than 50% of all microbes. This again illustrates that the overall composition and polymer architecture is important when designing materials with high biocidal efficacy.

Synthetic polymers

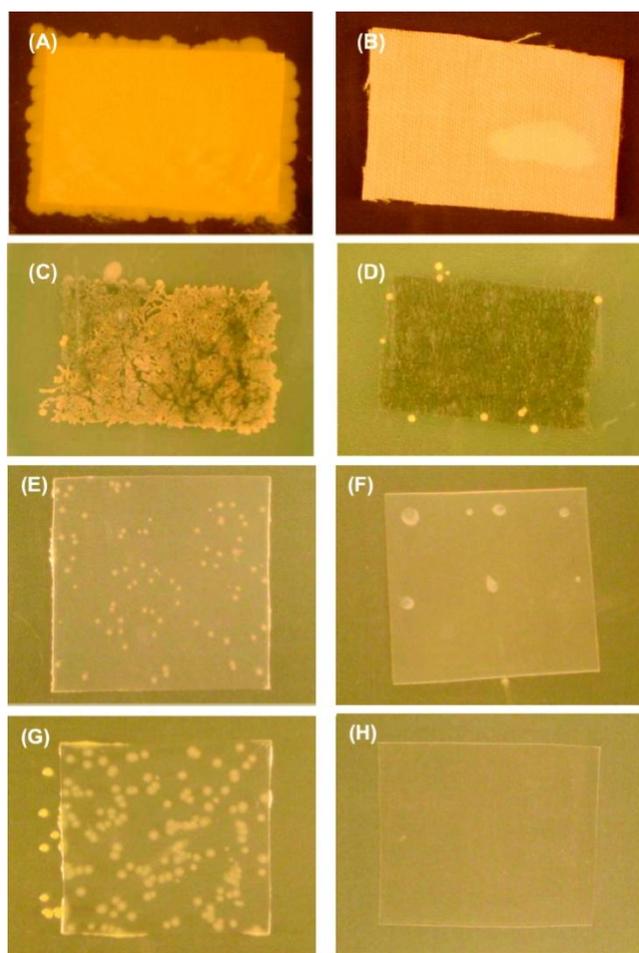


Fig 4 Digital pictures of the textiles and plastic substrates sprayed with *S. aureus*: (A) untreated cotton, (B) cotton spray-coated with 15 mg/mL BPAMP, (C) untreated polypropylene (nonwoven geotextile fabric), (D) polypropylene spray-coated with 15 mg/mL BPAMP, (E) untreated poly(vinyl chloride) substrate, (F) poly(vinyl chloride) substrate spray-coated with 15 mg/mL BPAMP, (G) untreated polyethylene substrate, and (H) polyethylene substrate spray-coated with 15 mg/mL BPAMP. Reprinted with permission from P. Dhende, S. Samanta, D. M. Jones, I. R. Hardin and J. Locklin, *ACS Appl. Mater. Interfaces*, 2011, **3**, 2830. Copyright 2011 American Chemical Society.

Polymers such as polypropylene and polystyrene have only alkyl and/or aryl functionalities and usually require harsh chemistry to produce synthetic “handles” that can be detrimental to the mechanical properties of the material. Our group has produced a Q-PEI with pendant benzophenone groups that allows the functionalization of *any material containing a C-H bond*.³⁰ Benzophenone can be excited with mild UV light (345-365 nm) to a diradicaloid triplet state that can abstract a hydrogen atom from a nearby C-H group, which creates a second carbon-centered radical. The two carbon radicals can then combine to form a new C-C bond.⁴⁰ We combined the photo-grafting ability of benzophenone with the biocidal activity of *N,N*-methyl-dodecyl Q-PEI to generate a benzophenone-containing antimicrobial polymer (BPAMP) that can be covalently attached to any surface that contains C-H or N-H bonds. After curing with 365 nm light, this coating renders a cross-linked network tethered to any plastic surface that serves as a permanent antimicrobial coating. To illustrate the wide applicability, we used BPAMP to coat cotton,

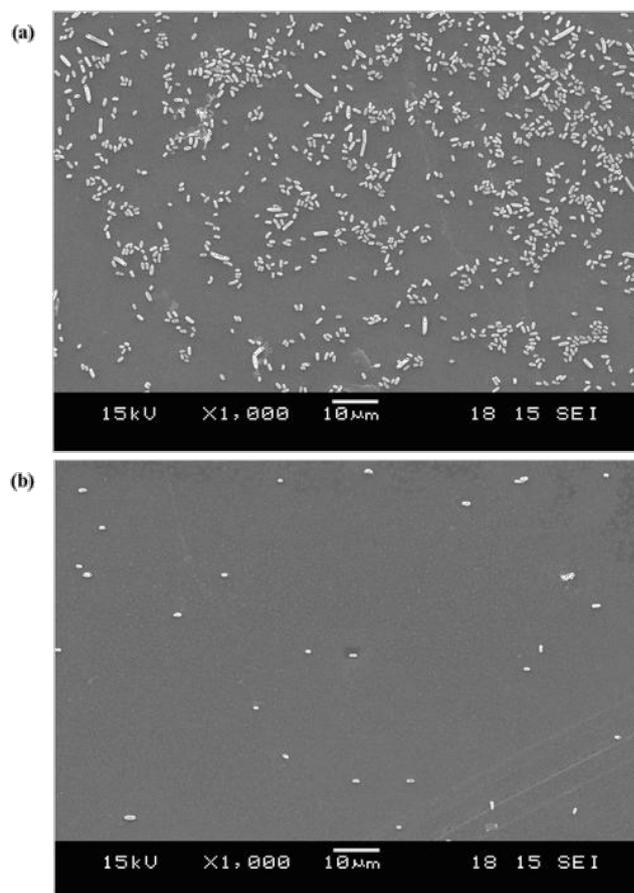


Fig 5 Scanning electron micrographs of (a) PET and (b) an alkylated P4VP film on PET after exposure to airborne *E. coli* subsequent incubation with solid growth agar for 24 h. Reprinted with permission from L. Cen, K. G. Neoh and E. T. Kang, *Langmuir*, 2003, **19**, 10295. Copyright 2003 American Chemical Society.

polypropylene, polyvinyl chloride, and polyethylene, which rendered these materials antimicrobial in a single functionalization step (Fig 4). A relationship between coating thickness and bacterial kill effectiveness of *S. aureus* and *E. coli* was noted, with films 35 nm or thicker killing essentially all bacteria applied. There are two plausible explanations for this behavior. If the alkyl chain intercalation or ion exchange mechanisms are valid, then perhaps the polymer film must be thick enough for free polymer ends to burrow past the peptidoglycan layer, interact directly with the phospholipid bilayer, disrupt ionic integrity, and cause cell death. Gram-negative bacteria generally have peptidoglycan layers of <10nm whereas Gram-positive bacteria usually have peptidoglycan layers of 20-80 nm.¹³ It is also possible that if the “phospholipid sponge” theory is valid, a film must be sufficiently thick to have enough storage volume to absorb sufficient anionic phospholipids to damage the bacterial membrane.

As a compliment to the “grafting to” approach with benzophenone that was employed by our group, Huang et al used the reactivity of benzophenone to attach an ATRP initiator to polypropylene in the form of a benzophenonyl 2-bromoisoobutyrate.⁴¹ The benzophenone end of the molecule was tethered to the surface by UV excitation, generating a polypropylene surface coated with ATRP initiator. PDAEMA

was then “grafted from” the surface using ATRP conditions that afforded several different molecular weights. The PDAEMA was then quarternized with ethyl bromide. As with Lee and Matyjaszewski’s previous antimicrobial coatings generated using SI-ATRP, a biocidal efficacy dependence on molecular weight was observed. In this system, biocidal efficacy correlated with the density of QA groups observed via the fluorescein dye method.⁴² Coatings with >9800 molecular weight polymer brushes, corresponding to a surface QA density >14 QA/nm², killed essentially all bacteria exposed to these surfaces.

Klibanov extended his earlier work that coated glass using PEI derivatives to strategies for coating cotton by adding a pendant photoactive (4'-azido-2'-nitrophenylamino)hexanoyl (ANPAH) group to branched, Q-PEI.⁴³ The aryl azide of the ANPAH group can undergo excitation upon exposure to UV light and form covalent bonds to cellulose. After coating a 2.5 cm² swatch of cotton with three layers of the ANPAH Q-PEI derivative, the swatch was effective in killing 4 x 10⁵ bacteria from a 10 mL solution. It is interesting that this work only described the functionalization on cotton, since aryl azides are known for similar photo-catalyzed nonspecific C-H insertion chemistries to benzophenone.^{44,45}

Another alternative and effective strategy is the use of plasma polymerization to derivatize inert polymer surfaces, such as polypropylene. Plasma polymerization generates a large amount of free radicals, which can also abstract a hydrogen from surface C-H bonds, and create a reactive carbon-centered radical which either terminates a nearby growing free polymer or initiates polymerization of a gas phase or aerosolized monomer. Wafa et al used this method to graft poly(glycidal methacrylate) (GMA) which was then reacted with HTCC.⁴⁶ Several other compounds were also used to functionalize polypropylene, including several cyclodextrin-entrapped molecules, but HTCC was the most effective, displaying a 1.33 log reduction for *E. coli* and a 1.30 log reduction for *S. aureus*. The authors noted that lower concentrations of HTCC corresponded to stronger antimicrobial activity in the case of *E. coli*. The same effect was previously documented by Lim⁴⁷ on HTCC functionalized cellulose, and was explained via a peculiar mechanism involving high surface concentrations of HTCC on a bacterial cell wall which formed a new barrier and prevented the leakage of cellular contents after the membrane has been disrupted by the polymer. An analogous approach was used by Wafa to also functionalize nylon 6,6.⁴⁸

The Kang group also used a plasma grafting approach to functionalize polyethylene terephthalate (PET) using three separate steps.⁴⁹ First, the PET surface was bombarded with argon plasma and exposed to air to create surface -OH and peroxy functionalities, from which P4VP was grown in a UV reactor. Finally, the pyridines were converted to alkyl pyridinium salts by the addition of hexyl bromide. The functionalized surfaces were tested against both air and waterborne *E. coli*. The surface demonstrated a strong ability to reduce bacterial contamination. The study also provided SEM images that showed any remaining bacteria were small in size (~1 μm compared to 2-6 μm for healthy bacteria), sparsely distributed, and did not appear to be actively reproducing or growing (Fig 5). Huh et al used a similar procedure,⁵⁰ except that acrylic acid was polymerized, then crosslinked by direct condensation with a quarternized chitosan derivative.

Inorganic surfaces

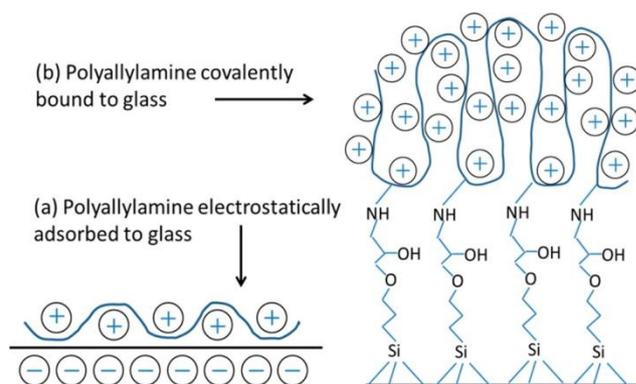


Fig 6. Morphology of a polycation that is electrostatically bound to glass versus one that is bound to the surface via silanes. Reprinted with permission from D. D. Iarikov, M. Kargar, A. Sahari, L. Russel, K. T. Gause, B. Behkam and W. A. Ducker, *Biomacromolecules*, 2014, **15**, 169. Copyright 2014 American Chemical Society.

Stainless steel

Direct functionalization of metals is an important application for antimicrobial functionalization, especially in the medical field where embedded implants, screws, and pins are a prime source of contamination. With conducting surfaces, electrografting is a well defined strategy for immobilizing organic molecules that contain a wide variety of functional groups.⁵¹ Electrografting has also been used to initiate polymerization from electrode surfaces. Ignatova et al used electropolymerization and ATRP to create hyperbranched antimicrobial polymers grafted from stainless steel (SS).⁵² The functionalization process begins with electropolymerization from the surface of SS using 2-(2-chloropropionate)ethyl acrylate (cPEA) as the monomer. ATRP was then employed using a secondary bromine containing monomer, resulting in a hyperbranched polymer with pendant alkyl halide groups. The alkyl halides were converted to pyridinium salts using pyridine. The group also created alkyl “-onium” salt coatings via electropolymerization of a monomer containing an N-hydroxy succinimide (NHS) ester. This NHS polymer was then reacted with branched PEI, creating a crosslinked PEI network polymer. The PEI was then alkylated with chlorooctane. Curiously, having created what appears to be a potent antimicrobial surface coating, the study only examines the bacterial adhesion properties of the coatings. Their results certainly suggested lower bacterial adhesion, with the poly(cPEA) having 10² fewer colony forming units (CFU) and the PEI derivative having 2.5x10³ fewer CFU than the control SS.

When functionalizing steel, corrosion prevention can be as high a priority as short-term antimicrobial efficacy. Biofilms speed up corrosion, so hybrid films that are both biocidal and corrosion preventing are a popular approach to functionalizing steel. An interesting combined antimicrobial/antifouling coating was established on SS by Yuan et al.⁵³ Poly(3-trimethoxysilyl)propyl methacrylate-b-poly(2-(dimethylamino)ethyl methacrylate) (PTMSPMA-b-DMAEMA) was fabricated via consecutive SI-ATRP, a procedure adapted from earlier work by the Matyjaszewski group⁵⁴. The pendant tertiary amino groups of the outer PDMAEMA block were quarternized with hexyl bromide to form an alkyl “-onium” salt. The inner PTMSPMA block containing trimethoxysilyl groups were hydrolyzed and condensed to form a cross-linked polysiloxane network, which

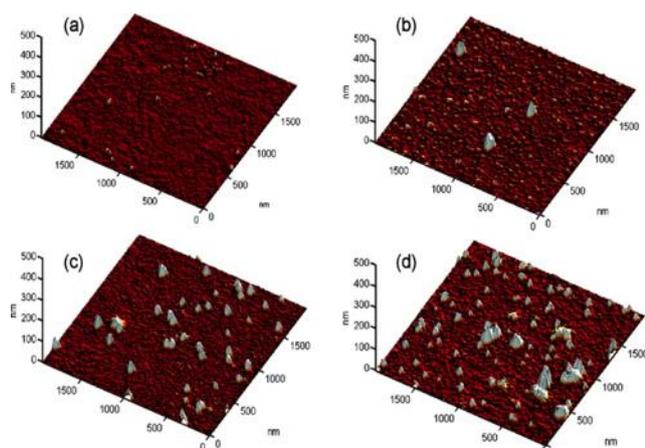


Fig 7 AFM images of glass surfaces after being immersed in the solution of PDMAEMA₉₇-PTMSPMA₆₀ in toluene ($C = 2.5$ g/L) for (a) 2.5 h, (b) 7.0 h, (c) 19 h, (d) 70 h at 70 °C. Reprinted with permission from J. Huang, R. R. Koepsel, H. Murata, W. Wu, S. B. Lee, T. Kowalewski, A. J. Russell and K. Matyjaszewski, *Langmuir*, 2008, **24**, 6785. Copyright 2011 American Chemical Society.

provided enhanced anticorrosion capability. A significant increase in corrosion potential and decrease of corrosion current compared to pristine SS in Tafel polarization curves and electrochemical impedance spectra demonstrated the anticorrosion capabilities of the material. The biocidal functionality of the coatings was also demonstrated with a reduction in viable cell counts on the surface from 10^6 cells/cm² to 10^3 cells/cm² when challenged with *D. desulfuricans*.

The same group also used layer-by-layer deposition of TiO₂ capped with an ATRP initiator to establish a “handle” for functionalization of stainless steel while simultaneously taking advantage of the antifouling capabilities of titanium.²⁸ SS was activated by washing with Piranha solution to create surface -OHs, which were then exposed to Ti(IV) tert-butoxide under inert conditions. The substrate was washed with water to generate surface -OHs on the titanium, and exposed to the Ti(IV) tertbutoxide for four more cycles before the addition of a reactive silane ATRP initiator. P4VP was polymerized from the initiator via ATRP, then alkylated with bromohexane. The alkyl-P4VP surface demonstrated a reduction in the viable cell count of *D. desulfuricans*, while simultaneously obtaining good anticorrosion properties via Tafel plot and electrochemical impedance spectroscopy measurements.

SiO₂

The functionalization of glass or silicon substrates with antimicrobial polymers has proven useful in probing the mechanism of biocidal activity and the effectiveness of various functional groups. In a single study, both the “grafting to” and “grafting through” motifs were examined by the Klivanov group on glass.¹⁷ Poly(4-vinyl-N-alkylpyridinium bromide) were covalently attached to pendant -NH₂ coated glass slides using two different strategies. In the “grafting through” method, -NH₂ functionalized glass were acylated using acryloyl chloride, followed by free radical polymerization of P4VP, and subsequent post-polymerization alkylation. In the “grafting to” method, the -NH₂ glass were reacted with 1,4-dibromobutane, which could

react with P4VP, along with subsequent alkylation of the bound

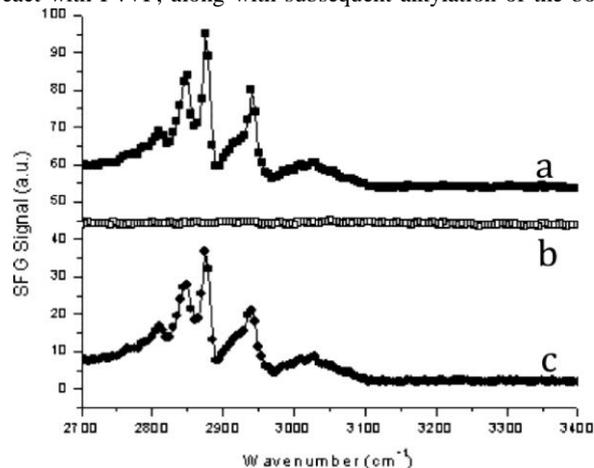


Fig 8 SFG spectra of the P2 coating surface (a) in air, (b) in contact with water, and (c) in air, after removal from water contact. Reprinted with permission from H. Han, J. Wu, C. W. Avery, M. Mizutani, X. Jiang, M. Kamigaito, Z. Chen, C. Xi and K. Kuroda, *Langmuir*, 2011, **27**, 4010. Copyright 2011 American Chemical Society.

polymer. Several different alkyl bromides were utilized for quaternization, with hexyl alkylated P4VP prepared by both methods demonstrating the highest bactericidal activity, killing over 94% of bacteria in a surface-aerosol test.

A notable example that stands out from hydrophobic “-onium” salts is work done by the Ducker group in which polyallylamine hydrochloride salt (PAA·HCl) was grafted to a silicon surface via a trimethoxy silane.⁵⁵ These amines are nonalkylated, and instead appear to derive their antimicrobial properties from the protonated amine functionality. This material was able to kill over 98% of *S. aureus* and *epidermidis* and 89% of *P. aeruginosa*, lending credence to the theory that alkylation is merely a method of balancing the hydrophobic/hydrophilic interactions, and that the cation is the prime mover of antimicrobial action. The group noted that it is possible to apply PAA·HCl to a glass surface and achieve only a physisorbed coating; however, this coating is not antimicrobial. They theorize that the positive charges of the material are interacting with the glass surface instead of being available to interact with bacterial cells walls. However, when the polymer is bound via a silane, the positive charges are more accessible and available for interaction, which leads to biocidal activity (Fig 6).

The Matyjaszewski group used ATRP to prepare a bifunctional block copolymer that contains a PTMSPMA surface anchoring segment and a PDAEMA biocidal segment.⁵⁴ “Grafting to” immobilization of the block copolymer was achieved by reaction between PTMSPMA and silanol groups on activated glass slides, and antimicrobial activity was achieved by quaternization of the PDMAEMA block with ethyl bromide. The effects of the grafting density of the PQA, polymer chain length, and the ratio of PDMAEMA to PTMSPMA on biocidal efficacy were investigated. It was demonstrated that surfaces with higher grafting density possessed higher biocidal activity, however there was no relationship between polymer chain length or block ratio on biocidal activity. The most critical factor was determined to be the density of QAs on the surface. In fact, a direct surface QA to bacterium ratio was established for this system, with about 10^{10} QAs being necessary to kill one *E. coli* bacterium. During these studies, a “patchiness” phenomenon was noted for these polymers

(Fig 7). The polymers aggregated and formed high density QA

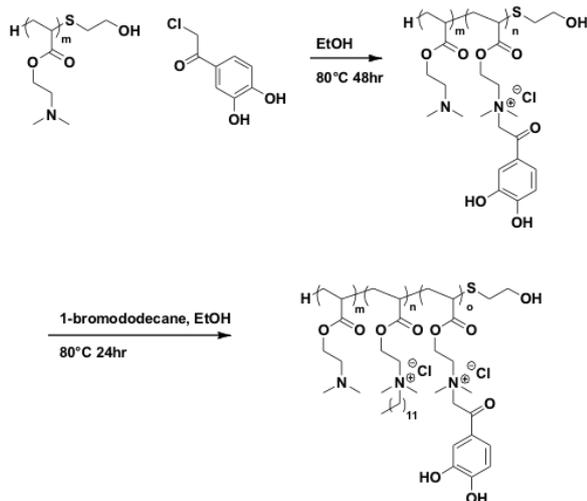


Fig 9 Synthesis of partially quaternized catechol antimicrobial polymer.

patches on glass which had superior biocidal activity. Furthermore, these patches were more effective than PQA grown via “grafting from” ATRP at the same overall charge density. This data implies that highly concentrated QAs over small areas are more effective than less concentrated QAs over a wider area.

The use of dopamine as a coating functionality is also very appealing because of its ability to coordinate via the catechol group to metals or adhere strongly to a variety of substrates via polydopamine networks. Dopamine has been used to coat metals such as silver,⁵⁶ metal oxides such as TiO₂,⁵⁷ silicon oxide,⁵⁸ and even highly inert surfaces like polyethylene and polytetrafluoroethylene.⁵⁸⁻⁵⁹ The Kuroda group used this potent attachment chemistry to coat a poly(2-(dimethylamino)ethyl methacrylate-co-methoxyethyl acrylate-co-dopamineacrylamide (PDAEMA-co-MEA-co-DMA) terpolymer onto glass, where the PDAEMA was quaternized with dodecyl chains.^{31a} After optimizing the ratio of these three monomers, a film was produced that killed nearly 100% of *E. coli* and *S. aureus*. Sum frequency generation spectroscopy (SFG) was used to study the polymer coated surface (Fig 8). The SFG spectrum indicated that in the dry state the dodecyl chains in the polymer aggregate at the surface. In solution, however, all surface organization is lost. This data indicates that the biocidal alkyl “-onium” functionality is presented at the surface in the dry state, not the MEA, catechol or polydopamine functionalities.

Kim et al used a different polymer system to establish the robustness of catechol attachment chemistry on a wide variety of surfaces.^{31b} Chain transfer polymerization was used to synthesize a poly(dimethylaminoethyl) acrylate (PDMA) homopolymer, which was partially quaternized with 2-chloro-3,4-dihydroxyacetophenone and bromododecane (Fig 9). Substrates consisting of Si wafer, titanium, quartz, Au, polystyrene, polyvinyl chloride, PET, polypropylene, and polycarbonate were soaked in a polymer solution for 24 hours at room temperature to allow for polymer attachment. The functionalized substrates were washed, and the presence of the polymer on the surface was confirmed via ellipsometry and contact angle measurements. The polypropylene surface was subjected to bacterial testing with *S. aureus* and *E. coli* to confirm the antimicrobial activity of the

polymer, with their testing showing near 100% killing efficiency. Furthermore, the coating was aged for 60 days at 60°C, and no loss in activity was observed.

Conclusions

In this feature article, we have highlighted the wealth of options available for permanent attachment of antimicrobial functionality to surfaces. Each tethering motif is highly effective in attaching to a complementary surface, but most of the approaches outlined above require multiple steps and are highly complex, which lead to challenges on the industrial scale. Techniques such as SI-ATRP or post-polymerization modification are likely too costly, time consuming, and have limited scalability for many high-throughput processes. While these approaches provide valuable insight into the mechanism of biocidal activity, most fall short of providing an optimized antimicrobial coating. The ideal coating should be synthetically accessible from low-cost starting materials and biocidal across a wide range of microbes and surface conditions, while remaining inactive towards mammalian cells. This material should also be stable under ambient conditions and be highly reactive towards the surface. It would also benefit from being non-specific, so that the same chemistry can be used across different surface chemistries.

In our opinion, another barrier to success in this field is the extreme heterogeneity of antimicrobial surface testing used by different researchers. For example, it is difficult to even compare the results of many of the studies described above, considering that they used different bacteria application methods, bacterial species, loading concentrations, solution versus aerosol application, application times, among other variables. Klibanov has initiated the standardization of this practice, and provided a method of testing antibacterial and antiviral activity of non-leaching flat surfaces which we strongly recommend.⁶⁰ Although this is an important step, standard methodologies for testing for other organisms such as fungi and three dimensional, non-leaching surfaces are still needed.

Finally, it is imperative that the mechanism of bactericidal action of these materials be more concretely determined in order to optimize interfacial design. Research has been performed on the hydrophobic/hydrophilic balance and its influence on surface antimicrobial efficacy, but this balance varies widely depending on molecular functionality and polymer architecture, and is highly qualitative in nature. There is also only a limited selection of research on the effects of these surface bound materials on fungi, viruses, and mammalian cells, a critical gap that needs to be addressed by the field. In our opinion, both Busscher’s adhesive forces model and the “phospholipid sponge” concepts especially deserve additional investigation, since the concepts appear promising by addressing the fact that solution and surface poly“-onium” antimicrobials appear to not be created equal.

Notes and References

We gratefully acknowledge financial support from the National Science Foundation (CAREER Award, DMR 0953112).

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