

Surpassing nature: rational design of sterile-surface materials

Kim Lewis¹ and Alexander M. Klibanov²

¹Department of Biology, Northeastern University, 360 Huntington Ave, Boston, MA 02115, USA

²Department of Chemistry and Division of Biological Engineering, Massachusetts Institute of Technology, Cambridge, MA 02139, USA

The rise of multidrug-resistant pathogens and recalcitrance of biofilm infections present a formidable challenge to combating infectious diseases. There are numerous disinfectants and antiseptics for treating materials in hospitals and community settings, and devices such as catheters impregnated with anti-infectives have been introduced into practice. However, there are many limitations of materials impregnated with a leaching antibacterial agent. Recently, non-leaching, permanent, sterile-surface materials have been developed in which one end of a long-chained hydrophobic polycation containing antimicrobial monomers is attached covalently to the surface of a material, for example, cotton or plastic. The polymeric chain allows the antimicrobial moieties to permeate into, and kill, the cells of the pathogen. These sterile-surface materials kill both air- and waterborne pathogens and are not susceptible to existing resistance mechanisms.

Introduction

We live surrounded by pathogens, and infectious diseases have been the main cause of mortality over millennia. The introduction of antibiotics in the 1940s promised to eradicate infectious diseases. Unfortunately, early successes in antibiotic development were followed by a daunting dual problem – the rise of resistant pathogens and a halt in novel antibiotic discovery. We are now confronted by *Staphylococcus aureus*, *Enterococcus faecalis* and *Mycobacterium tuberculosis* pathogens that are resistant to almost all currently available antibiotics [1,2]. Similarly, current measures against biofilm infections of indwelling devices are inadequate and the therapy of choice is often re-operation and removal of a prostheses or a catheter [3–5].

The last new class of broad-spectrum antibiotics (the fluoroquinolones) was discovered in the 1960s [6]. Culturable microorganisms, the source of most antibiotics, make up only ~1% of the total number of microbial species and their over-mining largely accounts for the end of the ‘golden era’ of antibiotic discovery [7]. Synthetic compounds thus far have failed to replace natural antibiotics, despite the combined efforts of genomics, combinatorial chemistry and high-throughput screening, because they are invariably pumped out across the outer

membrane barrier of gram negative bacteria by multi-drug-resistance pumps (MDRs) [8,9].

Encouraging recent developments should be noted – for example, a method for growing previously unculturable bacteria [10] and discovery of MDR inhibitors that might lead to dual-compound therapies based on a synthetic anti-infective and a pump inhibitor [8]. But for now we find ourselves close to where we started – in the pre-antibiotic era.

Successful pathogen counter-measures began not with systemic anti-infectives, but with the introduction of preventative public health measures a century ago. Countering the spread of infection has dramatically improved human health and increased longevity, surpassing the benefits of the subsequently introduced antibiotics [11]. At present, the spread of pathogens in hospitals has become a main cause of mortality from infectious diseases. Each year, ~90 000 people die in the USA alone from nosocomial infections [12] by pathogens such as ‘MRSA’ *S. aureus*. Attacking the spread of infection with novel technologies promises to stem both nosocomial and community-acquired diseases. Creation of materials lethal to pathogens will also address another unmet need – prevention of biofilm infections on indwelling devices.

A host of disinfectants, antiseptics and antibiotics has been developed to fight pathogens and biofouling with a leachable anti-infective usually incorporated into a polymeric surface coating [13]. This approach, however, suffers from problems – release of the active compound is temporary, a toxic substance leaches into the environment, and the gradually decreasing level of the released compound provides perfect conditions for resistance development. The ideal approach would create a permanently sterile, non-leaching material by covalently functionalizing its surface with an antimicrobial compound [14–21] and this emerging field is the subject of this article.

Designing sterile-surface materials

There is an obvious problem in designing a permanently sterile material – once attached to a surface, an antimicrobial molecule loses much of its mobility and, being unable to penetrate into the cell, becomes inactive. A possible solution is to link the antimicrobial agent to a long, flexible polymeric chain anchored covalently to the surface of a material.

Corresponding author: Lewis, K. (k.lewis@neu.edu).

Available online 26 May 2005

There are numerous antimicrobials suitable for immobilizing to a surface. Quaternary ammonium compounds (QACs) seemed attractive because their target is primarily the microbial membrane [22] and they accumulate in the cell driven by the membrane potential [23]. We initially chose *N*-alkyl-pyridinium as the antimicrobial moiety. To maximize efficiency, it was used as a monomeric link in the polymeric leash. Thus poly(4-vinylpyridine) (PVP) was selected as the carrying polymer and, following its *N*-alkylation, the resultant poly(4-vinyl-*N*-alkylpyridinium bromide) was attached covalently to a glass slide [14] (Figure 1).

To test the resultant antimicrobial properties we aimed to realistically emulate the spread of a nosocomial pathogen settling onto a surface from an aerosol created by a carrier [24]. Accordingly, a suspension of bacterial cells was sprayed onto a slide, followed by drying and application of nutrient agar. After an overnight incubation colonies formed on the surface by the surviving cells were counted.

Neither the parent PVP polymer attached to the glass slide surface nor its poly(4-vinyl-*N*-dodecylpyridinium) derivative affected bacterial survival. The slides had a milky appearance, suggesting the polymer chains were laterally interwoven owing to hydrophobic interactions (the 'spaghetti-bowl effect'), which overcame the electrostatic repulsion of the quaternary ammonium cations in the latter polymer. A variation in the length of the side alkyl chains between 0 and 12 produced some slides that were transparent and, importantly, exhibited excellent antimicrobial activities. For example, $\geq 99\%$ of all settled cells of *S. epidermidis*, *E. coli*, and *P. aeruginosa* were killed on contact with poly(4-vinyl-*N*-hexylpyridinium) [14], providing a validation for the concept of sterile-surface materials. These experiments also indicated that the immobilized polymeric chains must exist as individual entities (not entangled agglomerates) to ensure antimicrobial activity.

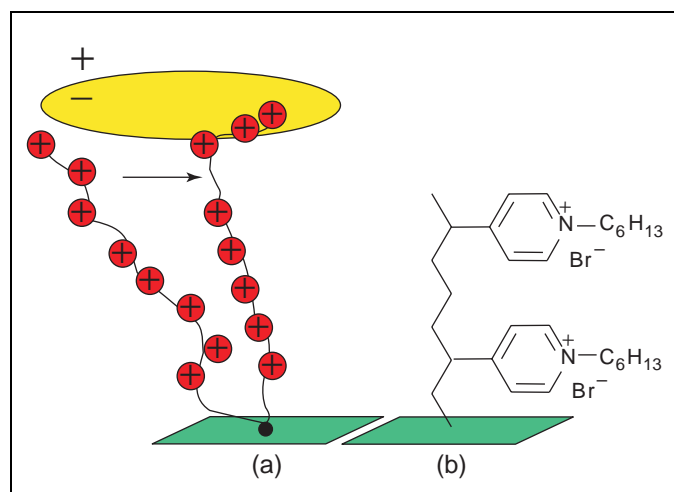


Figure 1. Design of a sterile-surface material. (a) The design rationale. An antimicrobial moiety is attached to a long flexible polymeric chain which, in turn, is attached to the surface covalently. The antimicrobial monomers are hydrophobic cations which can be actively 'electrophoresed' into the negatively charged cellular membranes of a microbial pathogen. (b) An example of a sterile-surface polymer with antimicrobial activity [14].

To explore whether the same general design for a sterile polymer could be attained with a different chemistry, we performed a structure–activity relationship analysis. A coating was created based on a polyethylenimine (PEI) instead of PVP backbone [17] (Figure 2a). This coating, however, was ineffective in making the glass surface bactericidal.

From our experience with PVP-based polycations, the most likely reason for the PEI coating's lack of activity was its insufficient hydrophobicity and/or positive charge. Therefore, we decided to increase both by *N*-alkylating the immobilized PEI with linear alkyl bromides of various lengths. This alkylation should not only make the polymer more hydrophobic but also raise its positive charge by converting PEI's primary, secondary and tertiary amino groups (some presumably already positively charged owing to the protonation at neutral pH) into permanently cationic quaternary amino groups. The *N*-alkylation of the PEI coating of a glass slide (Figure 2b) indeed gave rise to a pronounced bactericidal activity [17]. The subsequent *N*-methylation, raising the overall quaternary amino group density, boosted the bactericidal efficiency even further [17].

To elucidate the importance of the charge, we varied it while keeping the hydrophobicity roughly similar. To this end, instead of *N*-alkylating the immobilized PEI with hexyl bromide, we either *N*-acylated it with hexanoyl chloride or *N*-alkylated it with 6-bromohexanoic acid. These two immobilized polymers yielded non-bactericidal materials, even after the final methylation. These findings underscore the importance of positively charged polymeric chains enabling the molecule to stretch out.

Next, we used *N*-alkylated PEI to directly derivatize several common textiles [18]. The bactericidal activity of cotton, wool, nylon and polyester derivatized with

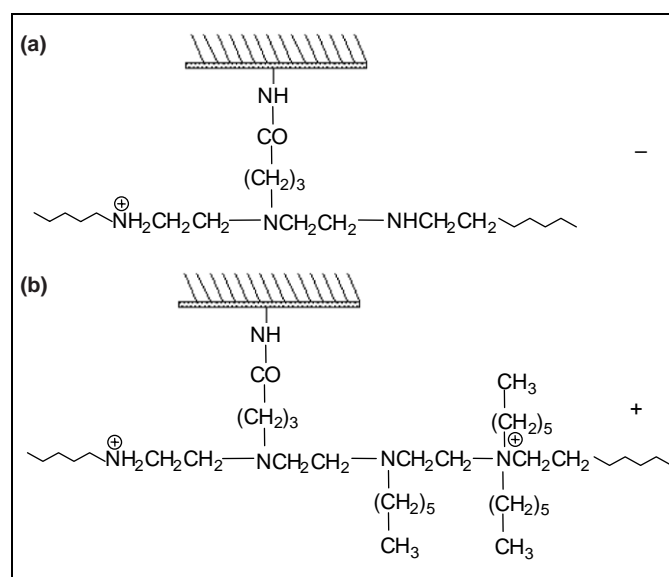


Figure 2. Dependence of polymer activity on hydrophobicity and positive charge. (a) Amino-glass slides *N*-acylated with 4-bromobutyl chloride, followed by attachment of high molecular weight polyethylenimine, PEI (a linear form of this branched polymer is shown for simplicity) produces an inactive polymer (–). (b) *N*-alkylation of (a) with linear alkyl bromide increases hydrophobicity and raises the positive charge, producing an active polymer (+) [17].

alkylated 750-kD PEI was tested against airborne *S. aureus*, *S. epidermidis*, *E. coli* and *P. aeruginosa*. All the derivatized fabrics were lethal to the bacteria tested – well over 90% of the deposited bacteria were invariably killed, with at least 98% killed in most instances. These bactericidal activities are similar to those observed for other, non-porous materials covalently modified with optimal *N*-alkylated PVPs or PEIs.

We also tested the effectiveness of the textiles with immobilized 750-kD alkyl-PEI against airborne fungal pathogens – a model organism, *Saccharomyces cerevisiae*, and a major human pathogen, *Candida albicans*. The fungicidal activities of the derivatized textiles were found to be nearly as high as their bactericidal activities.

Mechanism of action

Is the antimicrobial polycation really delivered into the cell of a pathogen? Varying the molecular weight of the immobilized polymer should directly test the hypothesis of antimicrobial mobilization by flexible polycations. To this end, *N*-alkylated PEIs of different molecular weights were covalently attached to amino-glass slides. Immobilized 750 kD and 25 kD PEIs were highly lethal to airborne *S. aureus*. By contrast, their 2 kD and 0.8 kD counterparts had negligible, if any, antibacterial activities, as did the immobilized and peralkylated PEI monomer analog 1,2-diaminoethane. Thus, to be bactericidal the immobilized polycation must have sufficiently long chains.

It should be noted that fatty alkyl chains attached to surfaces through quaternary amine anchors in several antibacterial materials reported in the literature ([25]; www.microbeshield.com) are far shorter than even the 0.8 kD PEI. Therefore, such materials cannot function via the same ‘hole-poking’ mechanism as those described by us [14,17–19,26], as well as possibly some others [27].

Two recent studies addressed another important question – does the sterile surface kill bacteria or merely inhibit their growth? In one study, a sterile surface was created by forming poly[2-(dimethylamino)ethyl methacrylate] on paper or glass, whereby the immobilized polymers appeared to be sufficiently long for traversing the ~30 nm cell envelope [20]. Incubating the modified materials with a suspension of *E. coli* or *B. subtilis* decreased the number of viable cells by several orders of magnitude. A ‘live or dead’ stain indicated that cells on the surface of modified paper rapidly lost their viability. The staining reports membrane integrity – the red fluorescing dye propidium iodide only penetrates into cells with leaky membranes. Hydrophobic cations, such as QACs are believed to kill cells by disrupting the membrane [22]. The live or dead test suggests that membrane damage is also the mechanism of action of sterile-surface polymers. These observations are consistent with ours, whereby glass slides modified with *N*-alkyl-PEI immersed in a suspension of bacteria reduced the live cell count by a factor of up to 10^9 , and no live cells were seen by the live or dead staining (Figure 3) [23]. Thus the *N*-alkyl-PEI modified surface appears to have the potential for completely killing all cells that come in contact with it.

Resistance

Resistance development by pathogens is the crucial limitation of existing antimicrobial agents and the main driving force behind the anti-infective drug discovery effort [2]. The most obvious application for sterile surfaces is to stem the spread of nosocomial diseases, such as those caused by the MRSA strains (note that although MRSA stands for ‘methicillin-resistant *S. aureus*’, these organisms actually carry plasmids conferring resistance to a whole slew of commonly used antibiotics [28]). We found that the sterile surface kills MRSA strains as effectively as the wild type [19].

A surface modified with *N*-alkyl-PEI and immersed in liquid apparently kills all bacterial cells encountered, as mentioned above [29]. This capability is essential in combating microbial biofilms. When a cell settles on a surface of indwelling devices such as catheters or prostheses, it can grow into a biofilm protected from the immune system by an exopolymer matrix [30–32]. Both planktonic populations and biofilms produce persister cells invulnerable to conventional antibiotics, explaining the recalcitrance of biofilm infections [4,33,34]. Persisters are dormant cells that overexpress Multidrug Tolerance (MDT) proteins that inhibit important cellular functions, such as translation [34]. Bactericidal antibiotics act by corrupting the target, leading to the formation of a toxic product that kills the cell. For example, aminoglycosides interrupt translation, leading to the production of truncated misfolded toxic peptides. By shutting down the target, MDT proteins prevent bactericidal antibiotics from corrupting their targets and killing the cell. A substance like *N*-alkyl-PEI is expected to damage the membranes in all bacterial cells, including persisters, and hence impart an important advantage to sterile surfaces in preventing biofilm formation.

A potential limitation for the use of hydrophobic cations is the presence of MDRs in all known bacteria and fungi [8]. Hydrophobic cations are the preferred substrates for MDRs, suggesting that the pumps evolved to combat this type of antimicrobial agent [35]. QACs are effective as disinfectants because they are applied at doses high enough to overwhelm the MDRs. However, their application is liable to the danger of increased resistance development through overexpression of the MDRs; additionally, there is a threat of developing cross-resistance to conventional antibiotics. In gram-negative species, broadly-specific MDRs of the RND family can extrude almost any antibiotic, and selection for increased resistance to QACs through overexpression of the pump will then also lead to increased resistance to such important antibiotics as tetracycline, azithromycin, and cipro [1]. An additional potential for cross-resistance development stems from the ‘neighborhood’ in which many MDRs are found. Several of them are encoded by mobile genetic elements, such as plasmids and integrons, which also contain resistance determinants against conventional antibiotics [36]. Selection for resistance to a QAC might lead to a rise in pathogens carrying this mobile element, resulting in increased antibiotic resistance.

It is instructive to consider how natural producers of hydrophobic cationic antimicrobials handle the problem of

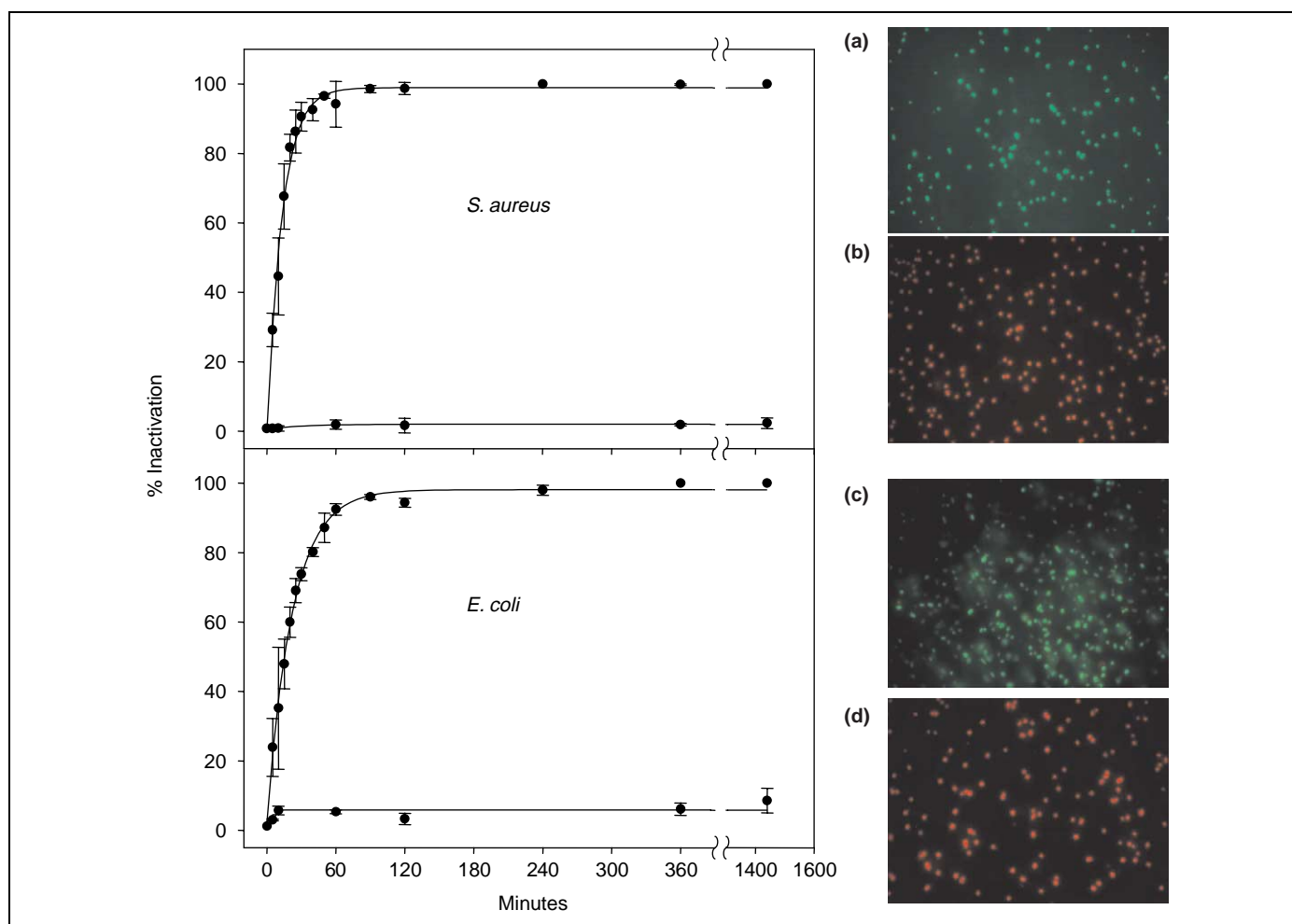


Figure 3. Time course of bacterial killing by a sterile surface. A bacterial suspension was exposed to the amino-glass surface derivatized with *N*-hexyl, methyl-PEI. Live or dead staining was used to monitor the appearance of dead (red) cells. Right panel, (a) and (c) represent a control, unmodified surface. Micrographs (b) and (d) were taken after a 2 h exposure of a cell suspension exposed to the derivatized material [29]. Reproduced, with permission, from [29].

pathogen drug resistance. Many common plants make berberine alkaloids, hydrophobic cations strikingly resembling synthetic QAC anti-infectives, such as ethidium bromide (Figure 4a). It appears that plants and chemists independently stumbled upon the same basic structure, and by essentially the same trial-and-error process.

Immobile and slow-evolving plants are confronted by rapidly evolving pathogens. It is easy to imagine that a mutation leading to the modification of a pathogen target will result in resistance to an antimicrobial produced by a plant. From this perspective, berberine seems a perfect anti-infective – its targets are apparently the membrane and DNA, into which it intercalates [37]. Neither can be ‘mutated’. The only way for bacteria to resist such a compound is by pumping it out via an MDR [35]. Plants developed an elegant solution to the resistance problem – they make berberine and methoxyhydrnocarpin, an MDR inhibitor that disables the resistance mechanism of pathogens and acts in synergy with the antimicrobial ([38]; Figure 4a).

Having a hydrophobic cation as the active component of the sterile polymer similarly presents a potential problem – resistance via microbial MDRs. However, we reasoned that they will fail to extrude a polymer because pumping out a single monomeric unit at a time should have little

effect because the rest of the polymeric molecule will still remain in the membrane (Figure 4b). We verified this idea experimentally by examining the activity of a surface modified with *N*-hexyl-PVP against a panel of *S. aureus* strains that consisted of a mutant deleted in the major NorA MDR; a wild type; and a wild-type strain carrying a QacA pump on a natural transmissible plasmid [19]. The material proved equally effective against all three strains. Similarly, the soluble analog poly(vinyl-*N*-methylpyridinium iodide) showed the same minimal inhibitory concentrations with all three strains (hexyl-PVP itself is insoluble in water). In a control experiment, the soluble monomer *N*-hexylpyridinium expectedly had the highest activity against the knockout mutant and the lowest against the strain expressing both the NorA and the QacA MDRs (Figure 4b). Finally, in a direct test we found that resistant mutants were not produced after repeated exposure of bacteria to a surface modified with *N*-alkyl-PEI [29].

These experiments demonstrate that the sterile-surface polymer is not subject to efflux by MDRs, the only known mechanism of resistance to hydrophobic cations. It appears that nature has not designed a similar material, which would explain why pathogens lack protection from it. It is unclear how resistance to a cationic hydrophobic polymer would develop. Although pathogens

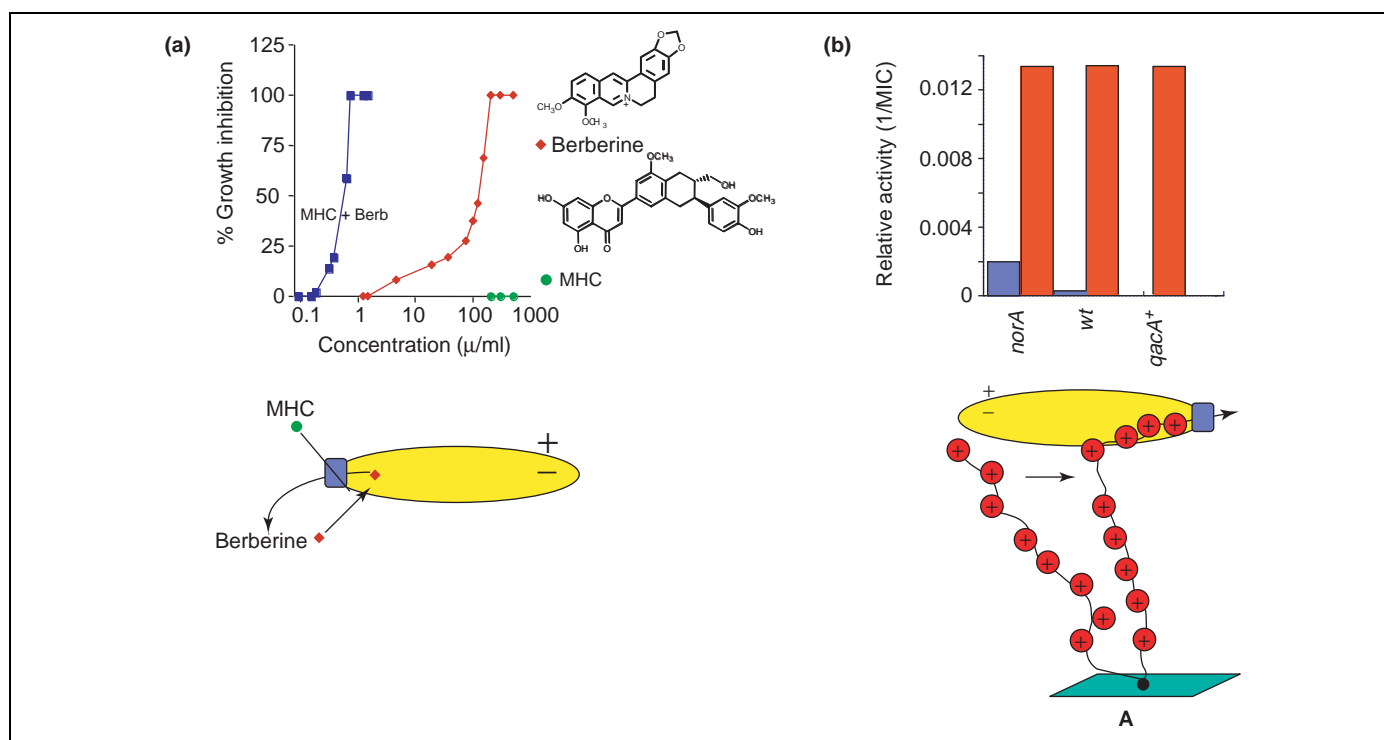


Figure 4. Countering pathogen resistance to hydrophobic cations – the plant strategy versus sterile surface. **(a)** Upper panel – plants produce berberine, a cationic antimicrobial that is potentiated by an MDR inhibitor 5'-methoxyhydrnocarpin (MHC) produced by the same plant (reproduced, with permission, from [38]). Berberine is a weak antimicrobial, whereas MHC is completely inactive. However, the combination of the two produces a potent antimicrobial. Lower panel – a model of berberine potentiation. Berberine is accumulated by the cell of a pathogen driven by the membrane potential, but is rapidly extruded by the MDR pump (blue). MHC inhibits the pump, enabling berberine to enter and kill the cell. **(b)** Upper panel – the activity of the monomeric *N*-hexylpyridinium (blue columns) is highest against a strain of *S. aureus* lacking the NorA multidrug pump, MDR, diminishes in the wild type and becomes unmeasurable in a strain additionally expressing a QacA MDR. Polymeric poly(vinyl-*N*-methylpyridinium) (red columns) is equally active against the same three strains. Lower panel – a model of a polycation penetration. An MDR pump (blue) can extrude one monomeric unit at a time, leaving most of the polycation in the membrane. The 'extruded' monomers will rapidly return into the membrane.

might still surprise us, for now the resistance-free status is a crucial advantage of sterile-surface polymers.

Conclusions and perspectives

The emerging area of non-leaching sterile surfaces has achieved several important milestones demonstrating the feasibility of this technology:

- (i) Proof-of-principle: surfaces modified with covalently attached polycations kill both airborne and waterborne microorganisms.
- (ii) Action spectrum: sterile surfaces kill a broad range of pathogens – gram positive and gram negative bacteria, as well as fungi.
- (iii) Mechanism of action: flexible polymers apparently reach across the microbial cell envelope, delivering the active moiety into the membrane and killing the pathogen. Only long-chained, moderately hydrophobic immobilized polycations exhibit microbicidal activity.
- (iv) Resistance development: the immobilized polycations are unique, and apparently have no analog in nature. They are not subject to existing mechanisms of resistance, such as MDR pumps or MDT cells, and no resistance develops upon repeated exposure to the polymer.

The next challenge is to create a simple, inexpensive and robust commercial process implementing the sterile-surface technology. The resultant materials are likely to be applied first in a hospital environment. Sterile linen, countertops, air ducts and other objects treated with a

sterile-surface polymer might provide an important barrier to the spread of nosocomial infections.

Microbicidal materials (prototypes described in this article) can turn out to be valuable in combating the all too real threat of biological warfare agents. An important attribute of our technology is that the treated objects function, look and feel the same as their non-derivatized and non-microbicidal predecessors. Moreover, derivitization with the antibacterial polycations can be combined with grafting other useful properties, as exemplified by making materials that are both microbicidal and water-repellent [15]. These, and all other, potential applications would greatly benefit if the bactericidal materials could also kill spores and viruses.

Household and every-day objects are another potential field of use. Currently, a large number of disinfectants are applied, creating both a hazard and a potential source for developing resistant pathogens. Our non-leaching sterile surface materials should have an obvious advantage.

A further challenge is to prevent formation of microbial biofilms. This raises an interesting question – can substances other than membrane-acting cations be delivered into, and kill, the cell? Cationic polymers are 'sticky' (albeit washable) and potentially toxic. However, significantly, we detected no toxicity of a surface modified by *N*-alkyl-PEI toward mammalian cells [29]. Finally, having more than one type of a sterile polymer is clearly advantageous, and different macromolecules could be created tailored to different applications.

Acknowledgements

Our studies reviewed herein were supported by NIH grants GM59903 and GM061162 (to K.L.) and by the U.S. Army through the Institute for Soldier Nanotechnologies under contract DAAD-19-02-D-0002 with the U.S. Army Research Office (to A.M.K.). The content does not necessarily reflect the position of the Government and no official endorsement should be inferred.

References

- Levy, S.B. and Marshall, B. (2004) Antibacterial resistance worldwide: causes, challenges and responses. *Nat. Med.* 10, S122–S129
- Lewis, K. *et al.* (2002) *Bacterial Resistance to Antimicrobials: Mechanisms, Genetics, Medical Practice and Public Health*, Marcel Dekker, New York
- Costerton, J.W. *et al.* (1999) Bacterial biofilms: A common cause of persistent infections. *Science* 284, 1318–1322
- Lewis, K. (2001) Riddle of biofilm resistance. *Antimicrob. Agents Chemother.* 45, 999–1007
- Parsek, M.R. and Singh, P.K. (2003) Bacterial biofilms: an emerging link to disease pathogenesis. *Annu. Rev. Microbiol.* 57, 677–701
- Walsh, C. (2003) Where will new antibiotics come from? *Nat. Rev. Microbiol.* 1, 65–70
- Osburne, M.S. *et al.* (2000) Tapping into microbial diversity for natural products drug discovery. *ASM News* 66, 411–417
- Lewis, K. and Lomovskaya, O. (2002) Drug Efflux. In *Bacterial Resistance to Antimicrobials: Mechanisms, Genetics, Medical Practice and Public Health* (Lewis, K. *et al.*, eds), pp. 61–90, Marcel Dekker
- Li, X.Z. and Nikaido, H. (2004) Efflux-mediated drug resistance in bacteria. *Drugs* 64, 159–204
- Kaerberlein, T. *et al.* (2002) Isolating “uncultivable” microorganisms in pure culture in a simulated natural environment. *Science* 296, 1127–1129
- Lederberg, J. (2000) Infectious history. *Science* 288, 287–293
- Weinstein, R.A. (1998) Nosocomial infection update. *Emerg. Infect. Dis.* 4, 416–420
- Danese, P.N. (2002) Antibiofilm approaches: prevention of catheter colonization. *Chem. Biol.* 9, 873–880
- Tiller, J.C. *et al.* (2001) Designing surfaces that kill bacteria on contact. *Proc. Natl. Acad. Sci. U. S. A.* 98, 5981–5985
- Lin, J. *et al.* (2003) Making thin polymeric materials, including fabrics, microbicidal and also water-repellent. *Biotechnol. Lett.* 25, 1661–1665
- Cen, L. *et al.* (2003) Surface functionalization technique for conferring antibacterial properties to polymeric and cellulosic surfaces. *Langmuir* 19, 10295–10303
- Lin, J. *et al.* (2002) Bactericidal properties of flat surfaces and nanoparticles derivatized with alkylated polyethylenimines. *Biotechnol. Prog.* 18, 1082–1086
- Lin, J. *et al.* (2003) Mechanism of bactericidal and fungicidal activities of textiles covalently modified with alkylated polyethylenimine. *Biotechnol. Bioeng.* 83, 168–172
- Lin, J. *et al.* (2002) Insights into bactericidal action of surface-attached poly(vinyl-N-hexylpyridinium) chains. *Biotechnol. Lett.* 24, 801–805
- Lee, S.B. *et al.* (2004) Permanent, nonleaching antibacterial surfaces. 1. Synthesis by atom transfer radical polymerization. *Biomacromolecules* 5, 877–882
- Waschinski, C. *et al.* (2004) Antimicrobial poly(oxazoline)s. *Polym. Prepr. (American Chemical Society, Division of Polymer Chemistry)* 45, 471–472
- Denyer, S.P. and Hugo, W.B. (1991) *Mechanisms of Action of Chemical Biocides: Their Study and Exploitation*, Society for Applied Bacteriology
- Severina, I.I. *et al.* (2001) Transfer of cationic antibacterial agents berberine, palmatine and benzalkonium through bimolecular planar phospholipid film and *Staphylococcus aureus* membrane. *IUBMB-Life* 52, 321–324
- Smith, S.M. *et al.* (1996) Survival of nosocomial pathogenic bacteria at ambient temperature. *J. Med.* 27, 293–302
- Isquith, A.J. *et al.* (1972) Surface-bonded antimicrobial activity of an organosilicon quaternary ammonium chloride. *Appl. Microbiol.* 24, 859–863
- Tiller, J.C. *et al.* (2002) Polymer surfaces derivatized with poly(vinyl-N-hexylpyridinium) kill airborne and waterborne bacteria. *Biotechnol. Bioeng.* 79, 465–471
- Morgan, H.C. *et al.* (2000) Method of creating a biostatic agent using interpenetrating network polymers. *US Patent No 6,146,688*
- Chopra, I. (2003) Antibiotic resistance in *Staphylococcus aureus*: concerns, causes and cures. *Expert Rev Anti Infect Ther* 1, 45–55
- Milovic, N.M. *et al.* (2004) Immobilized N-Alkylated polyethylenimine avidly kills bacteria by rupturing cell membranes with no resistance developed. *Biotechnol. Bioeng.* (in press)
- Leid, J.G. *et al.* (2002) Human leukocytes adhere to, penetrate, and respond to *Staphylococcus aureus* biofilms. *Infect. Immun.* 70, 6339–6345
- Jesaitis, A.J. *et al.* (2003) Compromised host defense on *Pseudomonas aeruginosa* biofilms: characterization of neutrophil and biofilm interactions. *J. Immunol.* 171, 4329–4339
- Vuong, C. *et al.* (2004) Polysaccharide intercellular adhesin (PIA) protects *Staphylococcus epidermidis* against major components of the human innate immune system. *Cell. Microbiol.* 6, 269–275
- Spoering, A.L. and Lewis, K. (2001) Biofilms and planktonic cells of *Pseudomonas aeruginosa* have similar resistance to killing by antimicrobials. *J. Bacteriol.* 183, 6746–6751
- Keren, I. *et al.* (2004) Specialized persister cells and the mechanism of multidrug tolerance in *Escherichia coli*. *J. Bacteriol.* 186, 8172–8180
- Hsieh, P.C. *et al.* (1998) Bacteria lacking a multidrug pump: a sensitive tool for drug discovery. *Proc. Natl. Acad. Sci. U. S. A.* 95, 6602–6606
- Sidhu, M.S. *et al.* (2002) Frequency of disinfectant resistance genes and genetic linkage with beta-lactamase transposon Tn552 among clinical staphylococci. *Antimicrob. Agents Chemother.* 46, 2797–2803
- Jennings, B.R. and Ridler, P.J. (1983) Interaction of chromosomal stains with DNA. An electrofluorescence study. *Biophys. Struct. Mech.* 10, 71–79
- Stermitz, F.R. *et al.* (2000) Synergy in a medicinal plant: antimicrobial action of berberine potentiated by 5'-methoxyhydronecarpin, a multidrug pump inhibitor. *Proc. Natl. Acad. Sci. U. S. A.* 97, 1433–1437

Reproduction of material from Elsevier articles

Interested in reproducing part or all of an article published by Elsevier, or one of our article figures? If so, please contact our *Global Rights Department* with details of how and where the requested material will be used. To submit a permission request on-line, please visit:

http://www.elsevier.com/wps/find/obtainpermissionform.cws_home/obtainpermissionform

Alternatively, please contact:

Elsevier Global Rights Department
Phone: (+44) 1865-843830
permissions@elsevier.com