

# Polycations. 23. Antimicrobial Surfaces for Prevention of Pathogen Transmission\*

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## Abstract

The continued evolution of bacterial and fungal species poses a significant difficulty for the treatment of disease of microbial origin. Given this situation, the prevention of transmission of such microbial diseases becomes of increasing importance. Efforts of this laboratory have been directed toward the destruction of microbial species on environmental surfaces as a prophylaxis toward infection, and we herein report on the efficacy of a system that demonstrates activity against both Gram-positive and Gram-negative bacteria, as well as fungi. We report specifically herein on the use of fabric materials so activated for the destruction of these microbial species, useful for a variety of surfaces within hospital and related settings wherein transmission of microbial disease is a major problem, while these approaches are also applicable for a variety of other types of surfaces.

## Keywords

Antimicrobial, Polycationic Organic Salts, Cell Disruption, Fabric, Environmental Surfaces, Prophylaxis

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## 1. Introduction

The evolution of bacterial species to generate resistance to classical antibiotics has become a major problem for the treatment of diseases of microbial origin [2]. As a result, *prevention of transmission* of these microbial diseases is of increased importance, particularly in a health service setting [3]. Thus, our laboratory has searched for approaches to reduce the threat of resistant bacteria through development of environmental surfaces that destroy the microbe in a passive manner (upon contact with the treated sur-

\*Please see reference [1].

face). Our emphasis has been particularly on fabric surfaces. Such modified fabric surfaces used in health care settings (modified linens, gowns, clothing, etc.) are critically important as they are active against a broad range of bacterial and fungal species, can be used multiple times (with proper laundering), and are relatively inexpensive to produce.

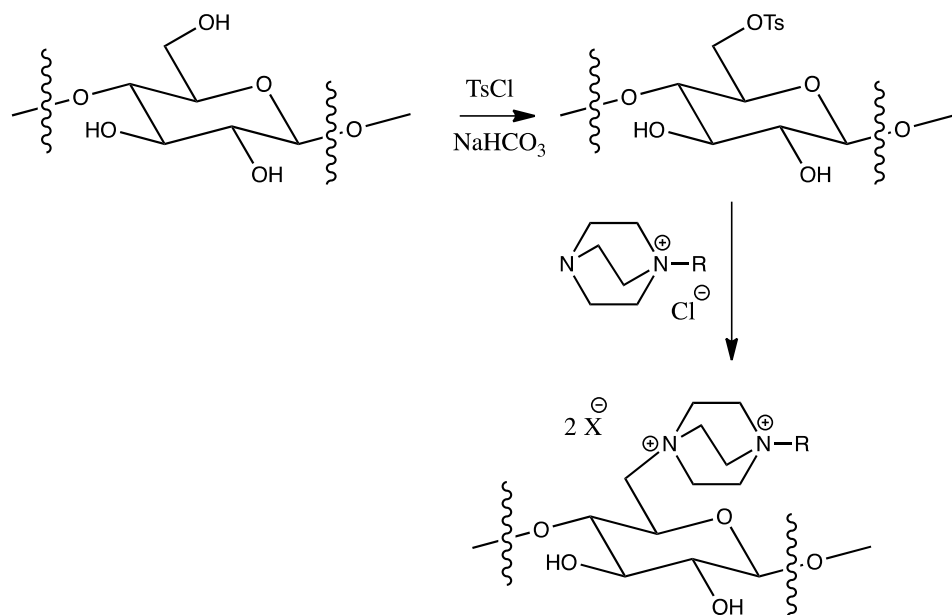
Specifically, we have developed several approaches for the permanent association of cationic lipids with fabric surfaces that would destroy a wide variety of microbial species that might come into contact with them. Additionally, these fabrics are antimicrobial to those microbial species that have undergone evolutionary mutations to generate resistance to classical antibiotic treatment. A method to destroy microbes before they have the opportunity infect the patient was the focus of our research. To this end we have developed and reported on several approaches toward the permanent attachment of cationic (polycationic) lipid species to fabric surfaces of several types resulting in antibacterial activity [4]-[11]. In addition to the antibacterial activity previously reported, we demonstrate an additional range of bacteria important in the healthcare section.

## 2. Results and Discussion

The fabrics investigated in this effort all bore functionalities that were capable of being modified by a two-step process of activation and functionalization, incorporating cationic lipid species as covalently attached entities. These fabrics specifically were carbohydrate based—**A** 100% cotton; **B** 50% cotton/50% nylon—or proteinaceous—**C** 100% silk; **D** 100% wool. With the carbohydrate fabrics activation of the pendant primary hydroxyl groups of the glucose residues was achieved by tosylation using tosyl chloride in the presence of sodium bicarbonate in aqueous/2-propanol medium, while the proteinaceous fabrics were similarly activated at the pendant primary hydroxyl groups of serine residues. Following activation, covalent attachment of the cationic lipid units were accomplished by nucleophilic displacement of the tosylate groups by reaction with the appropriate cationic lipid species, 1-alkyl-1-azonia-4-azabicyclo [2.2.2] octane halides. For a carbohydrate residue so activated and functionalized, the reaction scheme is shown in [Figure 1](#).

Upon drying in air, the resultant modified surfaces were suitable for testing for antimicrobial activity. All modified fabric surfaces were investigated using a standard procedure. This procedure involved:

Bactericidal activity was observed for *S. aureus*, as well as the microorganisms in [Table 1](#). After treated fabrics were incubated with bacteria or fungi and a lack of growth observed, a sample of the incubation medium was transferred to fresh growth medium and re-incubated (along with that from untreated samples as a control) to observe any residual growth of bacteria/fungi that were not killed by the treatment. The second incubations were found not to exhibit any noticeable growth of microbes, indicating that these fabrics were bactericidal.



**Figure 1.** Activation and functionalization of a carbohydrate-based fabric surface after washing with salt solution for association of desired anions.

**Table 1.** Microbial species killed by impinging on the modified surfaces—“Effective Modifications” indicate the length of carbon chains that provided kill of microbes.

Name	Gram Type	Effective Modifications	Source
<i>Staphylococcus aureus</i> (ATCC 35218)	Positive	C12-18	This work
<i>Staphylococcus aureus</i> (MRSA)	Positive	C12-16	[12]
<i>Staphylococcus aureus</i> (MSSA)	Positive	C12-16	[12]
<i>Bacillus anthracis</i> (spores)	Positive	C12-16	[13] [14]
<i>Bacillus cereus</i>	Positive	C12-18	This work
<i>Clostridium difficile</i>	Positive	C12-16	This work
<i>Burkholder cepacia</i>	Negative	C16	[12]
<i>Acinetobacter baumannii</i>	Negative	C16	[15]
<i>Klebsiella pneumonia</i>	Negative	C16	[12]
<i>Proteus mirabilis</i>	Negative	C16	[12]
<i>Proteus vulgaris</i>	Negative	C12-18	This work
<i>Pseudomonas aeruginosa</i>	Negative	C12 C12	This work
		C12/14 mix C12/16 mix C12 & C16	
<i>Escherichia coli</i>	Negative	C12/14 mix C12/16 mix C14/16 mix	This work
<i>Chaetomium globosum</i>	Fungus	C12-18	This work
<i>Saccharomyces cerevisiae</i>	Fungus	C12-18	This work
<i>Aspergillus niger</i>	Fungus	C12-18	This work
<i>Candida albicans</i>	Fungus	C12-18	This work

It was determined that the kill for all bacterial species investigated occurred in a very short period of time (<5 min) after addition to the modified surface. It was not possible to perform the experiments rapidly enough to make an exact measurement of time of kill.

The variety of bacterial and fungal species observed to be killed by contact with these surfaces is noted in [Table 1](#). Except for those so noted, these observations were made in this laboratory.

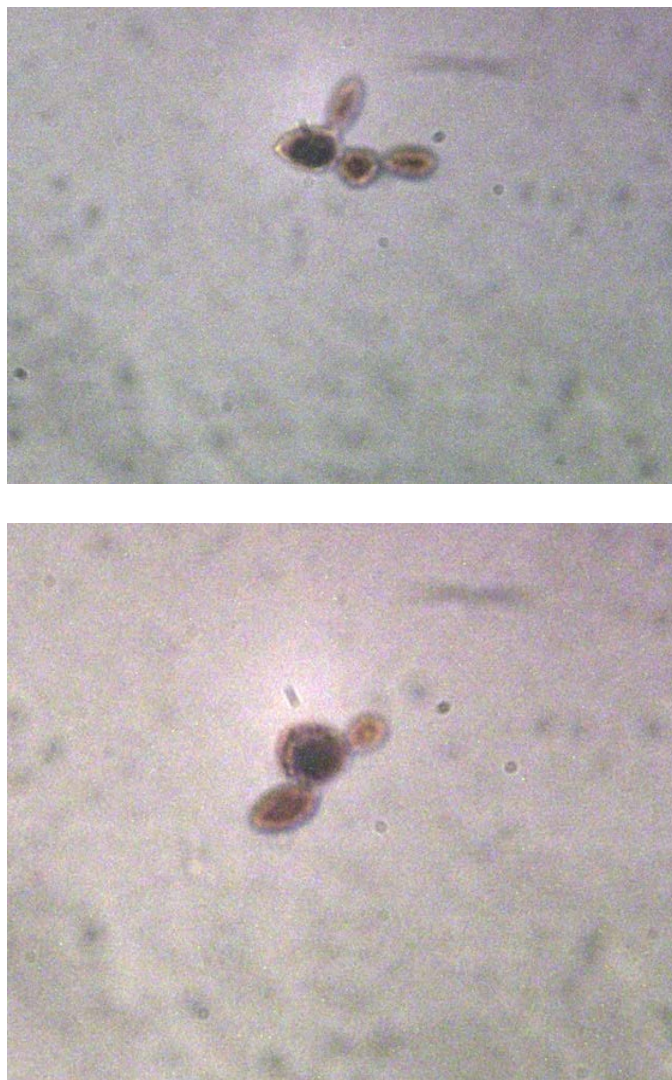
The kill of bacterial and fungal species is viewed as occurring rapidly by disruption of the cell. This was demonstrated using the Gram-positive bacterium *S. aureus* that had been treated with Gram stain after placement on the modified surface. Instead of retaining the crystal violet dye, it was released from the cell, destained and counterstained indicating disruption of the cell membrane. Similarly, this type of test was performed with *Saccharomyces cerevisiae* and shown below. Normal *S. cerevisiae* cells are Gram stained purple, as shown in [Figure 2](#) below.

In [Figure 3](#) are shown two examples of the cells after contact with a surface modified by attachment of a cationic lipid bearing a C16 chain. Cells have been destained and counterstained with the uptake of safranin, indicative of membrane disruption (intact cells would remain purple).

The modified fabric surfaces maintain their activity through multiple washings. Due to the physical nature of the kill, it is necessary to remove the build-up of dead cell debris from the treated surfaces for continued cell interactions with the active surface. Fabric samples have been washed under standardized conditions up to fifty times with an aqueous solution of non-ionic detergent and maintained full activity. The use of anionic detergents results in the detergent remaining associated with the surface and thereby inhibiting bactericidal activity.



**Figure 2.** *S. cerevisiae* cells stained purple with Gram stain—bright-field microscope, 1000× magnification.



**Figure 3.** Change in staining (safranin) of cells after contact with a surface modified by attachment of a cationic lipid with a C16 chain—bright-field microscope, 1000× magnification.

### 3. Materials and Methods

Modified fabric materials were prepared as has been previously reported [4] [5]. Fundamentally, strips of fabric were weighed and subjected to submersion in a 1/1 water/2-propanol solution (100 mL per gram of fabric used) containing an amount of *p*-toluenesulfonyl chloride and sodium bicarbonate, each equivalent to 10% of the mass of the fabric used. After immersion for 24 hours at ambient temperature ( $27^{\circ}\text{C} \pm 2^{\circ}\text{C}$ ) with constant agitation of the solution (by a magnetic stirrer—185 rpm) the fabric strips were removed, washed with ice/water, and reimmersed in a solution (1/1 water/2-propanol; 100 mL per g fabric used) containing 1-alkyl-1-azonia-4-azabicyclo [2.2.2] octane chloride (1 g per 10 g fabric). The solution was agitated by magnetic stirrer for 24 hours after which the fabric was removed, washed with ice/water, and allowed to dry



in the air. The modifications of the fabric samples were performed using salts bearing linear saturated alkyl groups of twelve, fourteen, and sixteen carbon atoms.

The 1-alkyl-1-azonia-4-azabicyclo [2.2.2] octane chloride salts were prepared by our previously reported method [4] [5] with linear saturated alkyl groups of twelve, fourteen, and sixteen carbon atoms, as illustrated in **Figure 4**. This process involved the reaction of 1 equivalent amount each of the appropriate 1-chloroalkane and 1,4-diazabicyclo [2.2.2] octane in ethyl acetate solution, 10 mL of ethyl acetate used for each gram of 1,4-diazabicyclo [2.2.2] octane used. After stirring at ambient temperature for 24 hours, the resultant precipitate was recovered by suction filtration, washed with ethyl acetate, and dried in air. As previously reported [4] [5] these materials exhibited  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra in accord with their proposed structures as well as quantitative elemental analyses.

Treated and untreated fabrics (1 cm  $\times$  1 cm) were incubated with  $1 \times 10^5$  bacteria (*S. aureus*) and the others shown in **Table 1** in 4 mL of Tryptic Soy Broth overnight at 37°C with shaking (100 rpm). The next day, 100  $\mu\text{L}$  of each sample were transferred to 4 mL of fresh growth medium and incubated overnight at 37°C with shaking. Samples were observed visually for growth.

The mechanism of bacterial (and fungal) kill is understood to be invasion of the outer membrane or cell wall by the lipid portion of the cationic lipid covalently attached to the surface through the cationic end, lipid being taken up until the cationic site impinges on the wall or membrane and causes an electrostatic disruption of that wall or membrane. A hole thus being generated in the bacterium (fungus) resulted in the cell contents being capable of being ejected. While a fully functionalized cotton surface would provide  $\sim 100,000$  such possible penetrations on a normal sized *E. coli* cell lying on such a surface, reductions in the amount of surface sites activated indicate that no more than 10 such invasions are necessary to kill such a cell. Thus, the method is quite efficient.

#### 4. Conclusion

The *prevention* of bacterial infection can go a long way toward ridding our health care facilities of diseases. Our approach is one that has been demonstrated to prevent the casual transmission of bacterial diseases among patients and health-care workers in such settings. Treatment of the surfaces on which bacteria (and fungi) can be transmitted from infected patient to those uninfected, using covalently attached cationic lipids, has been shown to kill bacteria and fungi on those surfaces within minutes. In this manner, the transmission of disease can be significantly prevented and a major source



**Figure 4.** Synthesis of 1-alkyl-1-azonia-4-azabicyclo [2.2.2] octane chlorides.

of disease eliminated.

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