Incorporating diffusible anti-microbial agents such as quaternary ammonium salts within the matrix of medical textiles is potentially problematic. Indeed, adverse effects such as allergic reactions may develop upon contact and diffusion of the exogenous agents into tissue regions that are exposed and weakened by injury or surgical intervention. These worrisome problems have largely been precluded by developing bacteriocins that are covalently immobilized to textiles such as cotton, as in the example of the agent AEM 5700. To our knowledge, however, the same approach has never been reported for single-use articles such as medical gauze, which seems unusual in that the active moiety would be non-articulating to skin and unable enter the bloodstream once assembled into the cotton as a polymer. It followed that testing the efficacy of immobilized quaternary ammonium syntheticals on single-use cottons was a logical progression in the development of specialized medical products, namely, self-sterilizing gauze to treat regions that have experienced open trauma in accident as well as surgical situations. In this preliminary work, the chemical modification of cotton bearing long-chain ammonium salts was achieved and its performance as an anti-microbial agent was characterized. Two syntheses were envisaged in which single-use cotton gauze could be chemically tailored to display anti-microbial activity. In the first approach, an intertwined organosilane-organic network bearing anti-microbial quaternary ammonium syntheticals was afforded by penetrating 3-trimethoxysilylpropyldecylammonium chloride, commonly known as AEM 5700, into the cotton matrix and polymerizing the monomers about the fibers. In a second approach, a related quaternary ammonium salt moiety was synthesized directly onto the solid-phase by employing a three-step synthesis consisting of tosyl group activation, amimation with long-chain aliphatic amine, and quaternization using iodomethane. Iodine-based colorimetric methods indicated that quaternary ammonium groups resided on the cotton whereas spectroscopic IR measurements clearly under detection thresholds implied the loadings must be low. The modified syntheticals were assayed for anti-microbial activity following their inoculation with gram negative bacteria by way of a procedure utilizing a GFP-synthesizing recombinant strain of Escherichia coli to facilitate visual observation. The modified syntheticals and respective controls were wetted with liquid culture and incubated at 37°C. Following incubation, control syntheticals afforded fluorescence, indicative of bacterial growth. A lack of fluorescence noted in modified syntheticals could not, however, be automatically equated to anti-microbial activity. To verify that the effect of the quaternary salt was realized through its anti-microbial action as opposed to a subtle mechanism such as the inhibition of protein expression, a secondary inoculation strategy was devised. Operating under the premise that bacterial growth on modified syntheticals stored at 4°C was negligible compared to death in the presence of ammonium salts, the control and modified syntheticals were applied to liquid Luria-Broth (LB) and LB-Ampicillin media and LB agar plates. In all cases, growth of bacteria was only noted in the native cottons, indicating that the low loading of ammonium groups was more than sufficient to impede bacterial growth. Plates inoculated with native cotton not only displayed GFP-fluorescence but also non-specific bacterial growth. In contrast, modified syntheticals afforded plates that were completely plaque-free under visible as well as UV light. It followed that the proposed syntheses could transform simple medical products such as gauze into materials that would facilitate topical therapies and long-term storage even under harsh conditions. The synthetic methods, principles and current state of this investigation are presented.

**Summary**

Two approaches were envisaged in which single-use cotton gauze could be chemically tailored to display anti-microbial activity. In the first approach, an intertwined organosilane-organic network bearing anti-microbial quaternary ammonium syntheticals was afforded by penetrating 3-trimethoxysilylpropyldecylammonium chloride, commonly known as AEM 5700, into the cotton matrix and polymerizing the monomers about the fibers. In a second approach, a related quaternary ammonium salt moiety was synthesized directly onto the solid-phase by employing a three-step synthesis consisting of tosyl group activation, amimation with long-chain aliphatic amine, and quaternization using iodomethane. Iodine-based colorimetric methods indicated that quaternary ammonium groups resided on the cotton whereas spectroscopic IR measurements clearly under detection thresholds implied the loadings must be low. The modified syntheticals were assayed for anti-microbial activity following their inoculation with gram negative bacteria by way of a procedure utilizing a GFP-synthesizing recombinant strain of Escherichia coli to facilitate visual observation. The modified syntheticals and respective controls were wetted with liquid culture and incubated at 37°C. Following incubation, control syntheticals afforded fluorescence, indicative of bacterial growth. A lack of fluorescence noted in modified syntheticals could not, however, be automatically equated to anti-microbial activity. To verify that the effect of the quaternary salt was realized through its anti-microbial action as opposed to a subtle mechanism such as the inhibition of protein expression, a secondary inoculation strategy was devised. Operating under the premise that bacterial growth on modified syntheticals stored at 4°C was negligible compared to death in the presence of ammonium salts, the control and modified syntheticals were applied to liquid Luria-Broth (LB) and LB-Ampicillin media and LB agar plates. In all cases, growth of bacteria was only noted in the native cottons, indicating that the low loading of ammonium groups was more than sufficient to impede bacterial growth. Plates inoculated with native cotton not only displayed GFP-fluorescence but also non-specific bacterial growth. In contrast, modified syntheticals afforded plates that were completely plaque-free under visible as well as UV light. It followed that the proposed syntheses could transform simple medical products such as gauze into materials that would facilitate topical therapies and long-term storage even under harsh conditions. The synthetic methods, principles and current state of this investigation are presented.

**Introduction**

Incorporating diffusible anti-microbial agents such as quaternary ammonium syntheticals within the matrix of medical textiles is potentially problematic. Indeed, adverse effects such as allergic reactions may develop upon contact and diffusion of the exogenous agents into tissue regions that are exposed and weakened by injury or surgical intervention. These worrisome problems have largely been precluded by developing bacteriocins that are covalently immobilized to textiles such as cotton, as in the example of the agent AEM 5700. To our knowledge, however, the same approach has never been reported for single-use articles such as medical gauze, which seems unusual in that the active moiety would be non-articulating to skin and unable enter the bloodstream once assembled into the cotton as a polymer. It followed that testing the efficacy of immobilized quaternary ammonium syntheticals on single-use cottons was a logical progression in the development of specialized medical products, namely, self-sterilizing gauze to treat regions that have experienced open trauma in accident as well as surgical situations. In this preliminary work, the chemical modification of cotton bearing long-chain ammonium syntheticals was achieved and its performance as an anti-microbial agent was characterized. Two syntheses were envisaged, examining diffusible moieties bearing similar modes of efficacy in light of their structural similarity. In the first method, AEM 5700 was applied onto the cotton gauze and cured thereon in a single step (Synthesis A). The anti-microbial action of AEM 5700-treated cotton was tested using a GFP-expressing strain of E. coli. In contrast, modified syntheticals afforded plates that were completely plaque-free under visible as well as UV light. It followed that the proposed syntheses could transform simple medical products such as gauze into materials that would facilitate topical therapies and long-term storage even under harsh conditions. The synthetic methods, principles and current state of this investigation are presented.
References

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Figure 6 shows that GFP was expressed on native gauze but not AEM 5700-treated gauze:
The photographs taken under UV light (in B&W and color) show that treating gauze with trimethoxysilylpropylmethyldiethacrylammonium chloride at least curbs the expression of GFP in gram (-) E.coli bearing the GFP gene. The question remaining to be answered is whether or not E.coli was actually killed by the action of AEM 5700. Inoculation method: Cotton was autoclaved for 30 min. Ampicillin resistant bacterial strain was inoculated with ampicillin (0.1mg/mL) and incubated at 37°C with 300 rpm shaking until A = 0.5, then diluted in LB+Amp. Native and modified cottons were put in liquid media and were incubated either at 4°C (results not shown) or 37°C (results, vide supra).

Conclusions
The quaternary amine strategy shows merit in the matter of producing improved single-use cottons and related self-sterilizing products of medical significance.

Background Material - The Bacterial Wall and Quaternary Ammonium Salts

Figures 9 and 10 illustrate the difference in wall structure of gram (+) and (-) bacteria. Gram positive bacteria have a thick peptidoglycan layer outside their plasma membrane. In contrast, gram negative bacteria have a thin peptidoglycan layer and an outer (lipid bilayer) lipid membrane (OM) containing lipopolysaccharide (LPS). Quaternary ammonium salts bearing long alkyl chains have the property of disrupting the delicate cell membrane and therefore do not need to be absorbed in solution to show their bactericidal effect.