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MicroShield 360 Research Overview

The following documents are a testament to the efficacy of the active ingredient in MicroShield 360 Antimicrobial. MicroShield has coupled this unique technology with a superior bonding agent that enables it's extended residual effect once electrostatically sprayed and bonded to a surface. We call this an "active" surface.

MicroShield 360 has been EPA approved for hundreds of facility types as well as hundreds of surfaces. Additionally, the MicroShield 360 Antimicrobial has been approved by the FDA for direct food contact surfaces which provides a testament to the not only the safety of the product, but it's strong bond to a treated surface which doesn't allow it to "leach" off into the surrounding environment.

MicroShield 360 provides a "broad spectrum" kill in that it neutralizes gram positive and gram-negative bacteria, certain viruses as well as fungi, algae, mold, yeast and spores. In effect, MicroShield 360 provides in uninhabitable surface for pathogens to live or grow on.

Please review the following documents and feel free to reach out to MicroShield 360 with further questions.

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Surface-Bonded Antimicrobial Activity of an Organosilicon Quaternary Ammonium Chloride

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The hydrolysis product of 3-(trimethoxysilyl)-propyldimethyloctadecyl ammonium chloride exhibited antimicrobial activity against a broad range of microorganisms while chemically bonded to a variety of surfaces. The chemical was not removed from surfaces by repeated washing with water, and its antimicrobial activity could not be attributed to a slow release of the chemical, but rather to the surface-bonded chemical.

For over a decade alkoxysilanes have been utilized by a number of industries as coupling agents to reinforce or impart desirable properties to a variety of materials. Plueddemann (9) has reviewed their use for such purposes. More recently Weetall and co-workers (13, 14) have described their use as coupling agents for insolubilizing enzymes on inert surfaces. A number of enzymes were found to remain biologically active when bonded to inorganic surfaces by alkoxysilanes. Their activity was shown to persist after repeated washing procedures. The immobilization of enzymes altered their activity as evidenced by changes in stability and *p*H optima. Hough and Lyons (5) have further advanced this technology with the successful demonstration of the coupling of enzymes to yeast cells. While this manuscript was in preparation, Venter et al. (11) reported the successful covalent coupling of catecholamines to glass beads resulting in biologically active particles.

3-(Trimethoxysilyl)-propyldimethyloctadecyl ammonium chloride (Si-QAC) is representative of a group of alkoxysilanes which have been under investigation in our laboratory over the past 3 years. This compound possesses antimicrobial activity when tested by a conventional serial tube dilution method. Similar alkoxysilanes in aqueous systems have been shown by Johannson et al. (6) to release methanol and form chemical covalent bonds with reactive surfaces (Fig. 1). The present study was conducted to determine whether the compound possessed antimicrobial activity when chemically bonded to a surface. This report was presented in part at the Annual Meeting of the American Society for Microbiology, Philadelphia, Pa., 23-28 April 1972.

MATERIALS AND METHODS

Chemicals. Si-QAC and ^{14}C-Si-QAC (labeled in the alkyl portion of the molecule) were prepared by J. R. Malek (Dow Corning Corp.) as methanolic solutions containing 50% solids (w/v) by a modification of the method of Speier et al. (10). Benzalkonium chlorides used in this work were alkylbenzyldimethyl ammonium chlorides (approximately 40% C_{12}; 50% C_{14}; 10% C_{16}) commercially available from Winthrop Laboratories, N.Y.

Organisms. Stock cultures of *Escherichia coli* B (ATCC 23226) and *Streptococcus faecalis* (ATCC 9790) were maintained on nutrient and Trypticase soy agar slants (Difco), respectively, at 4 C with monthly transfers. For preparation of inocula, *E. coli* B was transferred in nutrient broth for 3 consecutive days. *S. faecalis* was similarly transferred in Trypticase soy broth. The third 24-hr culture of each was harvested and washed in sterile saline by centrifugation at 15,000 rev/min for 15 min at 4 C in a Sorvall RC2-B centrifuge. Washed cell suspensions were resuspended in sterile saline to various optical densities (Coleman model 14 spectrophotometer) for aerosol inoculation of surfaces.

Mixed fungal spore suspensions of *Aspergillus niger* (ATCC 9642), *Aspergillus flavus* (ATCC 9643), *Aspergillus versicolor* (ATCC 11730), *Penicillium funiculosum* (ATCC 9644), and *Chaetomium globosum* (ATCC 6205) were prepared and applied as described in Military Standard-810B (8).

Test surfaces. Glass surfaces and cotton cloth were washed in detergent (Haemo-Sol) and rinsed exhaustively in tap water. Washed materials were then rinsed with 50% (v/v) isopropyl alcohol, followed by several distilled water washes before drying at 70 C for 30 min. Samples removed from the oven were allowed to stand at room temperature for 30 min under aseptic conditions prior to immersion in a bath of 0.1% Si-QAC or quaternary ammonium chloride (QAC) for 10 min. Chemically treated samples were again dried at 70 C for 30 min and allowed to remain

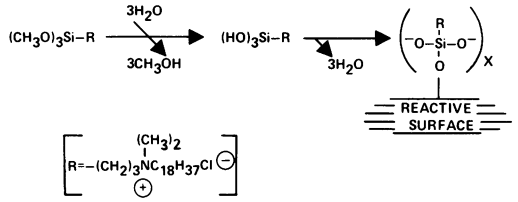

FIG. 1. *Hydrolysis and condensation of 3-(trimethoxysilyl)-propyldimethyl ammonium chloride with surfaces containing reactive functional groups.*

at room temperature in sterile petri dishes for 30 min before inoculation.

Radioactive cellulose acetate. This material was prepared by immersion of a 2-inch (ca. 5 cm) square of secondary cellulose acetate (four acetoxy groups per cellobiose unit) sheet, 1 mil (0.0254 mm) thick, without plasticizer or lubricant (Celanese Corp., Summit, N.J.), into a 0.1% solution of ^{14}C-Si-QAC in distilled water (specific activity 0.062 mCi/g), for 4 hr at 65 to 70 C. The square was then washed with two 20-ml portions of water. Further washing failed to remove radioactive material detectable by ^{14}C analysis of the sheet or of the wash water with a Packard Tri-Carb, model 3320 liquid scintillation counter. Samples of treated and untreated sheet (1 by 2 inches) were subjected together to aerosol contamination with *E. coli* B.

Bacterial test procedure. Treated surfaces plus untreated controls were subjected to aerosol inoculation in a polyvinyl chloride chamber designed for this purpose which was cylindrical and measured 47.5 inches (120.6 cm) in length by 11.8 inches (30 cm) in diameter. Test surfaces were placed on a wire support 6 inches (15.2 cm) from the bottom of the chamber, and the suspension of bacteria was aerosolized (average droplet size is less than 10 μm in diameter) onto the test surfaces by use of a pneumatic atomizer 1/4 J (Spray Systems Co., Wheaton, Ill.). Inoculated surfaces were held in sterile petri dishes at 25 C for 30 min before quantitation of viable organisms on the surface was accomplished by either wash recovery (1) or Rodac plate (2) techniques.

RESULTS

The antimicrobial activity of glass surfaces exposed to Si-QAC and QAC against *S. faecalis* is compared in Table 1. Recovery of 1,000 organisms from the control was used as a base line to calculate the reduction in the number of organisms caused by exposure of the glass surface to varying degrees, inoculated, and the reduction in surface contamination measured. Each sample was run in triplicate. On surfaces exposed to QAC, with no washing, 750 out of 1,000 organisms survived compared to two survivors on the Si-QAC-exposed surfaces. One 4-min wash permitted 100% survival of *S. faecalis* on the QAC-treated surface, indicating zero control of organisms. In contrast, after 50 washes, or 200 min, glass treated with Si-QAC effected a 95% decrease in surface contamination.

Cotton cloth identically treated (Fig. 2) was subjected to a mixed fungal spore suspension to demonstrate the relative antifungal activity of Si-QAC and QAC. Sample A is an untreated control. The zone of inhibition surrounding sample B, treated with QAC, demonstrates leaching of QAC. An identical sample, C, after

TABLE 1. *Survival of Streptococcus faecalis on glass surfaces*

No. of washes[a]	Viable organisms on surface[b]		
	No surface treatment	QAC surface treatment	Si-QAC surface treatment
0	1,000	750[c]	2
1	1,000	1,000	1
2	1,000	1,000	4
3	1,000	1,000	2
10	1,000	1,000	3
30	1,000	1,000	20
50	1,000	1,000	50

[a] Each wash consisted of 4 min under running tap water at 20 C.

[b] Standard error = ±10.

[c] Significantly different from the controls ($P < 0.05$).

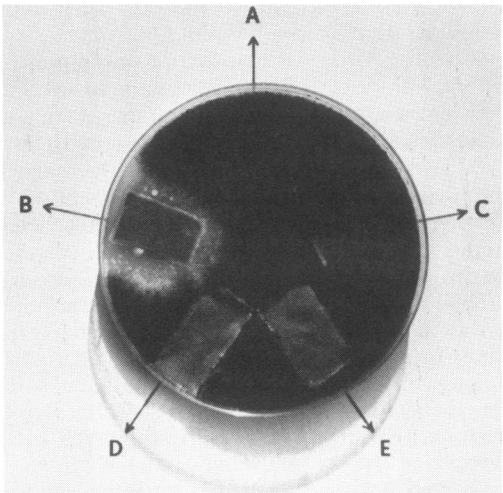

FIG. 2. *Antifungal protection of leached and non-leached cotton samples treated with: (A) untreated control; (B) QAC; (C) QAC plus 4-min water wash; (D) Si-QAC; (E) Si-QAC plus 4-min water wash.*

TABLE 2. *Durability of bonded ^{14}C-Si-QAC on cellulose acetate film to water washing and bacterial exposure*

Sample	Cellulose acetate treatment	*E. coli* B[a]	Wash[b]	Bacteria/ml	Disintegrations per min per ml (±S.E.)
Bacterial control	None	1,000	10 ml	980	27.5 (±2.52)
^{14}C-Si-QAC control	^{14}C-Si-QAC (10 μg)	None	10 ml	None	27.0 (±2.57)
Test sample	^{14}C-Si-QAC (10.2 μg)	1,000	10 ml	73	26.8 (±2.57)

[a] Samples exposed to aerosol inoculation followed by incubation in sterile petri dish at 37 C for 30 min; S.E. ± 12.

[b] Wash consisted of shaking sample in closed container with 10 ml of sterile distilled water at 150 rev/min for 15 min.

one 4-min wash under running tap water was not protected from fungal growth, indicating the easy removal of QAC. Both samples treated with Si-QAC (D, unwashed and E, washed) were not attacked by fungi. Further, the lack of a zone of inhibition around either sample D or E suggests no loss of chemical to the environment surrounding the sample.

^{14}C-Si-QAC-treated cellulose acetate sheet was used to gain further evidence of substantive antimicrobial activity of Si-QAC (Table 2). The bacterial control consisted of a 1-inch square of untreated cellulose acetate film, sprayed with *E. coli* B. The ^{14}C-control was the treated cellulose acetate without exposure to microorganisms, and the test sample was ^{14}C-treated cellulose acetate with *E. coli* B. After inoculation of the samples, all three were placed in sterile petri dishes at 37 C for 30 min. At the end of this time each film was placed in a vial containing 10 ml of sterile distilled water and shaken at 150 rev/min for 10 min, and samples of the water were taken for bacterial count and ^{14}C analysis.

Although the sample treated with Si-QAC effected a greater than 92% reduction in *E. coli* compared to the bacterial control; no loss of ^{14}C-labeled material from the cellulose acetate film due to either the wash procedure or exposure to microorganisms was detected.

To detect release of Si-QAC below the level of radioisotope analysis, a bioassay was used (Fig. 3). Tube A was a bacterial growth control. All tubes used in this experiment contained 10 ml of sterile broth. The broth was inoculated with 0.1 ml of a 24-hr culture of *E. coli* B and was incubated at 37 C for 24 hr on a rotary shaker at 150 rev/min, and plate counts were made. Tube B contained a 1 by 2 inch cellulose acetate sheet with approximately 10 μg of ^{14}C-Si-QAC bonded to the surface as measured by ^{14}C analysis. Tube C contained 10 μg of Si-QAC added to the broth or that amount which would be released from the cellulose acetate in tube B

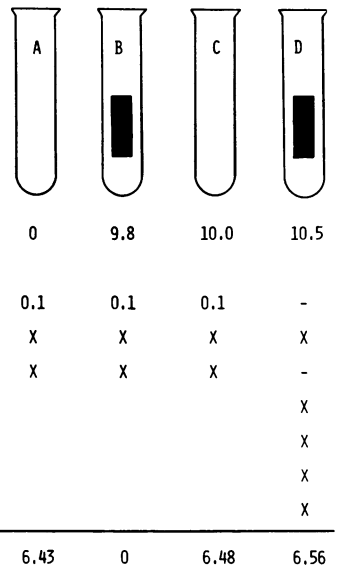

FIG. 3. *Bioassay determination of Si-QAC release from chemically treated cellulose acetate (CA) film samples.*

if 100% of the Si-QAC were leached into the broth. Both tubes B and C were inoculated, incubated, and counted in an identical manner to tube A. It can be argued that any activity due to slow release of material from the cellulose acetate film in tube B may not be chemically comparable to the direct addition of Si-QAC to the broth as in tube C. Tube D was thus included to control this variable, i.e., a treated cellulose acetate film identical to that used in tube B, but uninoculated for 24 hr and incubated under the same conditions. At the end of this time, the film was removed, and the remaining broth was inoculated, incubated, and counted. The broth exposed to the treated Si-QAC film for 24 hr was not antimicrobial. Thus, both the radioactive and bioassay data indicate that Si-QAC is an effective antimicrobial agent when firmly bonded to a surface,

and its activity is not dependent on slow release of the compound.

A number of substrates (Table 3) were found to exhibit durable antimicrobial activity when treated with Si-QAC, against a spectrum of microorganisms (Table 4) of medical and economic importance.

DISCUSSION

Vol'f (12) reported that nitrophenylacetate, nitrophenylphosphate, and "certain other agents" formed covalent bonds with polyvinyl alcohol fibers. The resultant fibers acquired a relatively permanent antibacterial (staphylococci, intestinal bacillus) antifungal (candida, trichophyton, epidermophyton), and antiprotozoal (trichomonas) activity. Vol'f states, however, that to achieve this activity the bond between the fiber and the active agent must be weakened by heating the fiber in a moist state or by washing in detergents of an acid nature to allow transportation (diffusion) of these groups within the microbial cells. In a similar manner Davis (3) attempted to produce antimicrobial surfaces through the slow release of hexachlorophene from polyethylene plastic surfaces.

The evidence presented indicates that the organosilicon quaternary ammonium salt we have investigated does not have to enter the cell for bactericidal activity. Although the antimicrobial activity of quaternary ammonium salts was first extensively examined by Domagk (4) in 1935, the specific site of action of this class of compounds is still being investigated. Their activity has been attributed by various workers to membrane phenomena, i.e., membrane lysis, membrane enzyme inactivation, or interference with ion transport (7). By chemical analogy, our data would lend support to the thesis that the site of action may be the membrane or cell wall, but probably not intracellular organelles.

The biological activity of Si-QAC bonded to surfaces may offer a method of surface protection without addition of the chemical to the environment. The treatment of solutions (serum, water, etc.) by passage over Si-QAC-

TABLE 3. *Si-QAC-treated substrates exhibiting antimicrobial activity*

Siliceous surfaces	Man-made fibers	Metals
Glass	Acrylic	Aluminum
Glass wool	Modacrylic	Stainless steel
Sand	Polyester	Galvanized metal
Stone	Cellulose acetate	
Ceramic	Rayon	Miscellaneous
	Acetate	Leather
Natural fibers	Anidex	Wood
Cotton	Spandex	Rubber
Wool	Vinyl	Plastic
Linen	Dacron	Formica
Felt	Viscose	

TABLE 4. *Microorganisms susceptible to Si-QAC*

Bacteria (gram positive)	Algae
Staphylococcus aureus	*Cyanophyta* (blue-green) *oscillatoria*
Streptococcus faecalis	*Cyanophyta* (blue-green) *anabaena*
Bacillus subtilis	*Chrysophyta* (brown)
	Chlorophyta (green) *Selenastrum gracile*
Bacteria (gram negative)	*Chlorophyta* (green) *Protococcus*
Salmonella chloeraesius	
Salmonella typhosa	Fungi
Escherichia coli	*Aspergillus niger*
Mycobacterium tuberculosis	*Aspergillus flarres*
Pseudomonas aeruginosa	*Aspergillus terreus*
Aerobacter aerogenes	*Aspergillus verrucaria*
	Chaetomium globosum
Yeast	*Penicillum funiculosum*
Saccharomyces cerevisiae	*Trichophyton interdigital*
Candida albicans	*Pullularia pullulans*
	Trichoderm sp. *madison* P-42
	Cephaldascus fragans

treated surfaces without chemical adulteration of the solution also appears feasible. The use of alkoxysilanes of this nature may be useful in defining the site and mechanism of action of antimicrobial agents.

ACKNOWLEDGMENTS

We thank J. R. Malek for necessary chemical synthesis. We are also indebted to W. Schar and H. Taskier of Celanese Plastics Corporation, Summit, N.J., for supplying the cellulose acetate film used in this study. The guidance and suggestions of D. R. Bennett and J. L. Speier were of great value throughout these studies.

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Evaluation of the effect of MicroShield 360 on biofilms of *Listeria monocytogenes*

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The University of Georgia

Introduction:

Scientists have recently discovered that bacteria can communicate with each other. When confronted with environmental conditions that are unfavorable, such as refrigeration, they signal each other. Imagine riding in a convertible with some of your friends and it begins to rain. One passenger would say "let's put the top up". Bacteria do the exact same thing. They signal one another to begin forming a protective coating called a "biofilm". For the food processor, this is disasterous because once the bacteria form this protective coating, it is nearly impossible to kill them. In fact, studies have demonstrated that when spraying sanitizer on bacterial colonies encased in biofilms, only 60% of the bacteria die. This is less than a 1 log reduction. Where this becomes a problem is in foods that are fully cooked or ready to eat. In coolers, freezers, and on processing belts and other equipment surfaces used to process or store chicken nuggets, chicken patties, hot dogs, cheese, frozen pizza, deli meats, and many other foods, if these bacteria are allowed to form biofilms, then a major problem occurs. By visiting the USDA-Food Safety Inspection Service's website (http://www.fsis.usda.gov/OA/recalls/recdb/rec2000.htm), it is easy to see how many recalls were performed in the year 2000 (this is the most current year they have listed). It is astonishing to see the number of companies affected by *Listeria*, *E. coli* O157:H7, and

Salmonella recalls. These recalls, in some cases, involve hundreds of thousands of pounds of meat products. In fact, the second case listed on this site refers to a recall of Cargill's ready to eat turkey and chicken because of *Listeria* contamination. In this single instance, a total of 16,895,000 lbs. were recalled at a market value average of $6-$7 per pound (**$109,817,500**). This is one example of many pages of recalls listed on this page of the website. In all cases, *Listeria* contamination of meats involves a process by which *Listeria* from drains or floors is transmitted to cooling units in a cooler or freezer or to equipment surfaces as the result of aerosolization of the bacteria when employees use high pressure hoses to spray floors. The bacteria then attach to the surfaces of the cooling units or equipment, signal each other, and begin to form biofilms. When this happens, the sanitation crew cannot fully clean and sanitize the plant because their sanitizers are not able to penetrate the biofilm and get down to the bacteria. When fully cooked, ready to eat products are then produced the next day, *Listeria* are blown onto them in the cooler or freezer from the cooling units or are transferred by contact with a contaminated belt. The product then is evaluated by a laboratory, found to be positive, and the product is recalled or someone gets sick and 30% of these individuals will die as a result of this contamination. Scientists have demonstrated, using the most high tech methods available, that *Listeria* is almost impossible to eliminate from a processing plant once it is established.

MS360 is an antimicrobial that controls bacteria not by poisoning the cell, but by puncturing the cell wall on a microscopic level unlike chemical sanitizers. The MS360 technology relies on an electrically-charged spiked molecular structure. In nature, most microbes carry the opposite electrical charge from the molecule in MS360. Therefore, the

bacteria are physically and irresistibly drawn into contact with MS360 pointed molecules, to the point that the cell walls are punctured and destroyed. MS360 does not leach or mutate. MS360 is EPA registered. Once it is sprayed on the surface, it bonds and retains its effectiveness indefinitely unless painted, covered, or worn off in high-traffic areas. The coating makes surfaces easier to clean and is based on the same technology as Rain-X or Dr. Sholl's Odor Destroyers. It is important that, before application of MS360, the surface should be cleaned. And once applied, the surface should be kept as clean as possible with mPerial and the microfiber cloth. Theoretically, application of MS360 to a surface, such as the stainless steel wall of a cooler would greatly assist in preventing *Listeria monocytogenes* producing a biofilm on the surface and causing a post-cook contamination scenario.

The purpose of this study was to determine if electrostatic application of MS360 to stainless steel coupons could prevent the formation of a biofilm of *Listeria monocytogenes* on that surface. If successful, this technology would be extremely useful for poultry and red meat producers to prevent massive product recalls.

Procedures:

1) 15 stainless steel coupons were treated by electrostatic application of MS360, coating the entire coupon on both sides. 15 stainless steel coupons were not treated to serve as controls.

2) These coupons were allowed to remain in a bag for 3 weeks before any testing was done to determine if the MS360 had an antimicrobial impact after 3 weeks time as advertised.

3) All 30 coupons were dipped into actively growing *Listeria monocytogenes* (multiple strain mixture)

4) The *Listeria* were encouraged to form a biofilm by placing the coupons (individually in different containers) into minimal media. This signaled the

Listeria "you have no food and are in danger" but did not kill them. The *Listeria* then began to produce the biofilms.

5) After 12 hours, the coupons were removed from the minimal medium and the biofilms (if present) were recovered.

6) The coupons were placed into sterile urine sample cups with sterile glass beads and shaken to remove the biofilms from the surface.

7) This sample was plated onto Modified Oxford Agar to recover *Listeria*.

8) The *Listeria* was counted and the groups (treated with MS360 vs. controls) were evaluated statistically.

9) The experiment was conducted 3 times. (3 Repetitions)

Results:

The ability of MS360 to prevent the attachment and biofilm formation by *Listeria monocytogenes* on stainless steel three weeks after being applied to the coupons was impressive. Photos 1 and 2 show turbidity and a *Listeria* biofilm covered coupon for the untreated sample. Photos 3 and 4 show the stainless steel coupon in the minimal medium with no visible biofilm on the MS360 treated coupon and no turbidity in the medium, indicating little if any growth.

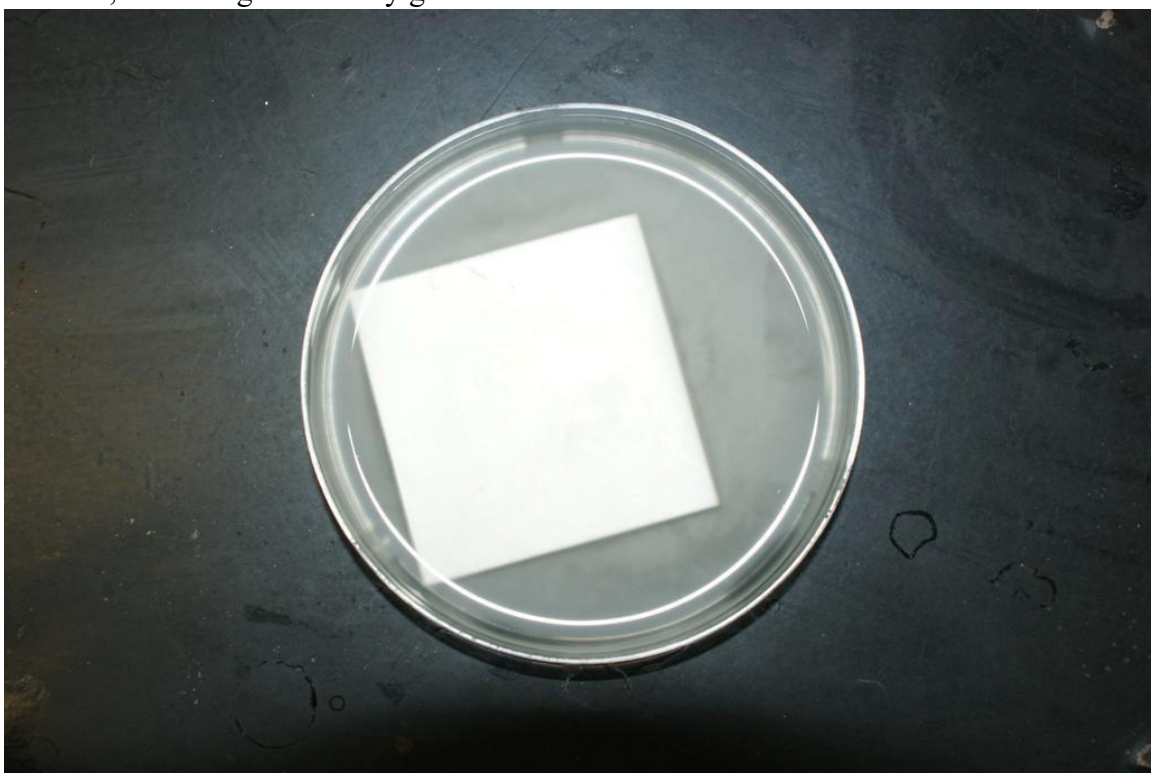


Photo 1. Stainless steel coupon control (untreated), showing significant *Listeria* growth and biofilm formation.

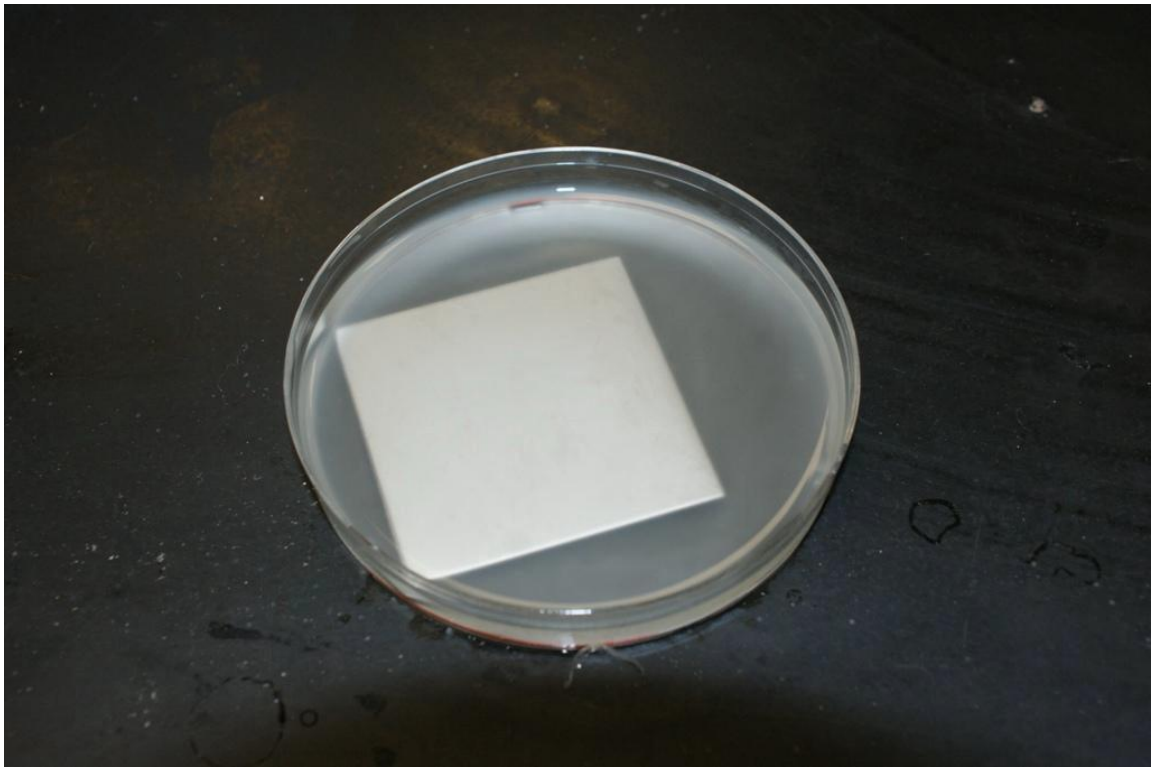


Photo 2. Stainless steel coupon control (untreated), showing significant *Listeria* growth and biofilm formation.

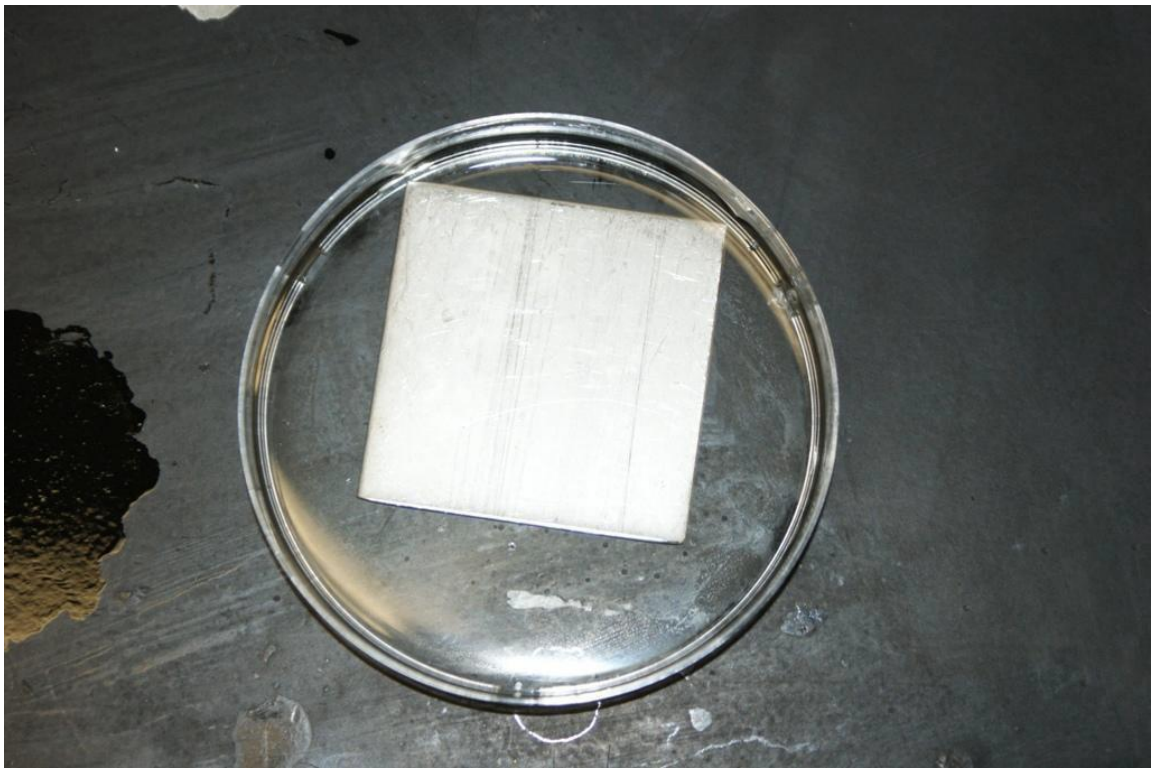


Photo 3. Stainless steel coupon treated with MS360, showing no *Listeria* growth or biofilm formation.

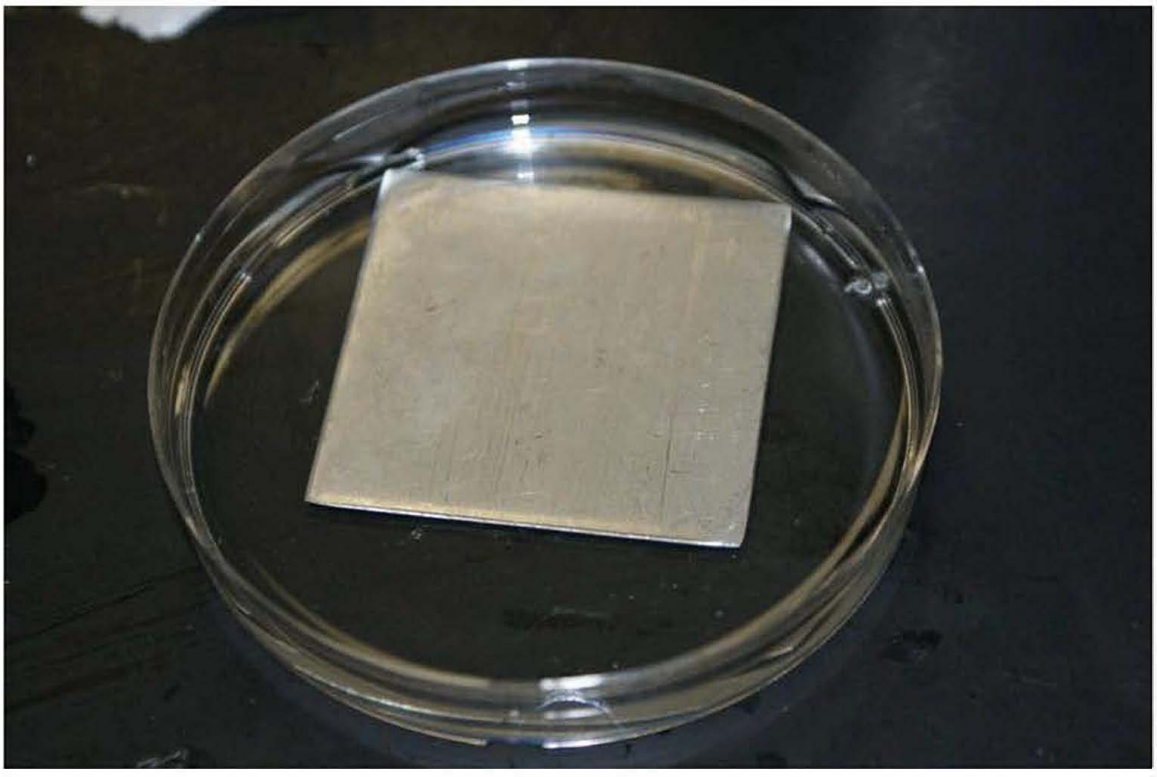
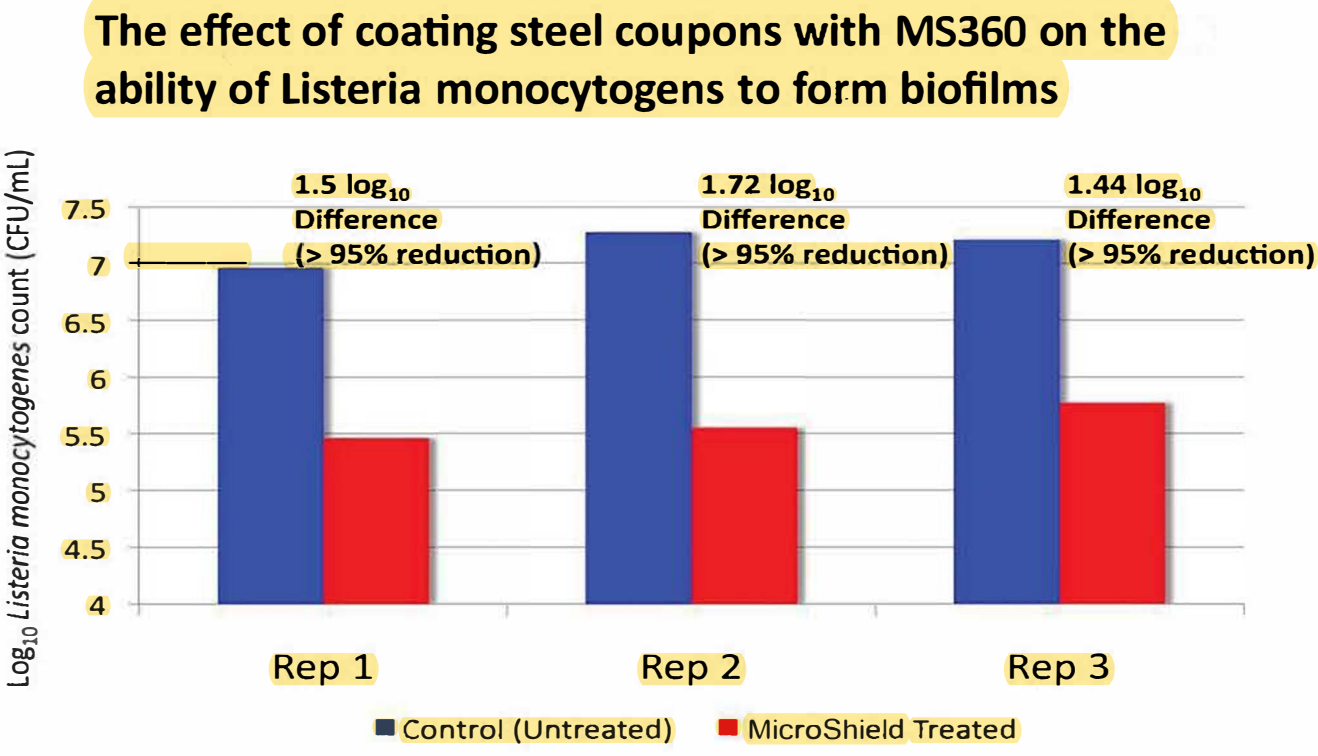


Photo 4. Stainless steel coupon treated with MS360, showing no *Listeria* growth or biofilm formation.

The data obtained in this study may be found in Appendix A (raw data) and summarized in Figure 1.



In repetition 1, coating the stainless steel coupon significantly ($P \leq 0.05$) lowered *Listeria monocytogenes* numbers recovered after attempting to form a biofilm by 1.5 $\log_{10}$ (>95%) colony forming units (CFU)/mL of recovery rinse. In repetition 2, *Listeria* was lowered by 1.72 $\log_{10}$ (>95%) and in repetition 3, *Listeria* was lowered by 1.44 $\log_{10}$ (~95%). It is important to note that in this experimental trial, extremely high numbers of *Listeria* were used to challenge the ability of MS360 to perform (~1,000,000 cells/0.1 mL of inoculum placed into solution with each coupon). In the real world, only a few *Listeria* cells would be traveling on a fomite (such as a dust particle) and would be transmitted to the surface. Theoretically, the effect of MS360 would be much greater in terms of preventing biofilm formation by *Listeria* on stainless steel surfaces when the bacterial challenge is much lower than was used in this experiment. For a study such as this, a reduction of 1 $\log_{10}$ would be considered of practical significance and would be highly useful in an industrial setting for prevention of *Listeria* biofilms in food processing facilities and, thereby, preventing extremely costly recalls.

It is important to note that three weeks elapsed between the time when the MS360 was coated onto the coupons and when the coupons were exposed to bacteria, demonstrating a powerful residual effect. Also, MS360 was not only able to control *Listeria* growth and biofilm formation on the coupon, but was also able to prevent growth of *Listeria* in the surrounding medium, as indicated by the photos. MS360 represents a significant step forward in terms of allowing food processors to meet the zero tolerance requirement of USDA-FSIS for fully cooked foods such as deli meats, hot dogs, and chicken nuggets. This product would be very useful for companies that wish to prevent *Listeria* biofilm formation in their facilities.

Appendix A: Raw data of *Listeria monocytogenes* recovered from stainless steel

coupons after allowing them to form biofilms for treated (MS360) versus untreated (controls).

MS360 Coupon Coating Study

Sample	Rep	Count	Dilution	Actual Count	Log10
C1	1	61	5.00	6100000	6.785329835
C2	1	66	5.00	6600000	6.819543936
C3	1	50	5.00	5000000	6.698970004
C4	1	88	5.00	8800000	6.944482672
C5	1	56	5.00	5600000	6.748188027
C6	1	92	5.00	9200000	6.963787827
C7	1	46	5.00	4600000	6.662757832
C8	1	29	6.00	29000000	7.462397998
C9	1	15	6.00	15000000	7.176091259
C10	1	21	6.00	21000000	7.322219295
					6.958376868
C11	2	116	5.00	11600000	7.064457989
C12	2	15	6.00	15000000	7.176091259
C13	2	24	6.00	24000000	7.380211242
C14	2	57	6.00	57000000	7.755874856
C15	2	24	6.00	24000000	7.380211242
C16	2	101	5.00	10100000	7.004321374
C17	2	29	6.00	29000000	7.462397998
C18	2	84	5.00	8400000	6.924279286
C19	2	16	6.00	16000000	7.204119983
C20	2	25	6.00	25000000	7.397940009
					7.274990524
C21	3	15	6.00	15000000	7.176091259
C22	3	16	6.00	16000000	7.204119983
C23	3	90	5.00	9000000	6.954242509
C24	3	33	6.00	33000000	7.51851394
C25	3	20	6.00	20000000	7.301029996
C26	3	16	6.00	16000000	7.204119983
C27	3	20	6.00	20000000	7.301029996
C28	3	24	6.00	24000000	7.380211242
C29	3	69	5.00	6900000	6.838849091
C30	3	15	6.00	15000000	7.176091259
					7.205429926

Sample	Rep	Count	Dilution	x20 CM sq	Count	Log10
T1	1	17	3.00	340	340000	5.531478917
T2	1	18	3.00	360	360000	5.556302501
T3	1	52	2.00	NA	5200	3.716003344
T4	1	18	3.00	360	360000	5.556302501
T5	1	22	3.00	440	440000	5.643452676
T6	1	18	3.00	360	360000	5.556302501
T7	1	24	3.00	480	480000	5.681241237
T8	1	26	3.00	520	520000	5.716003344
T9	1	48	3.00	960	960000	5.982271233
T10	1	25	3.00	500	500000	5.698970004
						5.463832826
T11	2	161	3.00	NA	161000	5.206825876
T12	2	19	3.00	380	380000	5.579783597
T13	2	60	3.00	NA	60000	4.77815125
T14	2	30	3.00	600	600000	5.77815125
T15	2	12	3.00	240	240000	5.380211242
T16	2	40	3.00	800	800000	5.903089987
T17	2	21	3.00	420	420000	5.62324929
T18	2	14	3.00	280	280000	5.447158031
T19	2	40	3.00	800	800000	5.903089987
T20	2	42	3.00	840	840000	5.924279286
						5.55239898
T21	3	42	3.00	840	840000	5.924279286
T22	3	49	3.00	980	980000	5.991226076
T23	3	55	3.00	NA	55000	4.740362689
T24	3	29	3.00	580	580000	5.763427994
T25	3	25	3.00	500	500000	5.698970004
T26	3	48	3.00	960	960000	5.982271233
T27	3	44	3.00	880	880000	5.944482672
T28	3	36	3.00	720	720000	5.857332496
T29	3	32	3.00	640	640000	5.806179974
T30	3	52	3.00	1040	1040000	6.017033339
						5.772556576

Interaction of Infectious Viral Particles with a Quaternary Ammonium Chloride (QAC) Surface

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The antiviral activity of a surface-bonded quaternary ammonium chloride (QAC) was examined in this study. The mechanism of inactivation was elucidated by a combination of infectivity assay, radioactive labeling assay, and sedimentation analysis. Although the virions are still infectious when attached onto the chemically modified surface, we found these viruses are inactivated if they are eluted from the surface. The inactivation is caused by the disruption of the viral envelope with subsequent release of the nucleocapsid. No evidence indicates the released nucleocapsid is further disrupted. An enveloped virus shows a much higher affinity for the QAC-treated surface than a nonenveloped one due to hydrophobic interaction. The QAC-treated beads can effectively remove the enveloped viruses at low protein concentrations. The titer of herpes simplex virus was reduced by a factor of nearly 5 logarithm units in a 0.5 wt % bovine serum albumin solution with less that 10% protein loss. However, the presence of proteins in the solution reduced both the rate and capacity of this nonspecific adsorption–inactivation process. As a consequence, the removal efficiency is relatively poor in solutions with high protein content.

INTRODUCTION

Quaternary ammonium chlorides (QAC) are cationic surface-active agents with antimicrobial activity.[1,2] The major mode of action of QAC was identified as the cytolytic damage caused by its effects on cellular permeability.[3] The virucidal capacity of a QAC-Zephiran (alkyl-dimethylbenzylammonium chloride) against various types of viruses was summarized by Klein and Deforest.[4] They reported that Zephiran can effectively inactivate lipid-containing viruses like vaccinia virus, some nonlipid viruses such as reovirus, and bacteriophages but is not effective against smaller nonlipid viruses such as picornaviruses. QAC display their antimicrobial activity even after being covalently immobilized on inert supports because they can act on the membranes of various cells. The effects of surface-bonded organosilicon QAC on bacteria, yeast, fungi, and algaes have been the topic of several papers.[3,5,6–8] The antimicrobial activity of immobilized QAC is not due to leaching of the compound since the radiotracer study by Isquith[3] showed no leakage of the immobilized QAC from the surface. Our own cytotoxicity tests also confirmed this point. However, the antiviral activity of these immobilized compounds has not been investigated previously.

In the current study, we have demonstrated the efficacy of QAC-treated surfaces to remove an enveloped virus, herpes simplex virus type-1, in aqueous solutions with and without the presence of proteins. Experiments have further demonstrated that the viruses were inactivated. The mechanism that underlies this inactivation is elucidated by using doubled-labeled HSV-1.

MATERIALS AND METHODS

Chemicals

3-(Trimethoxysilyl)propyldimethyloctadecyl ammonium chloride (Si-QAC), Dow Corning 5700 antimicrobial agent, was provided by W. Curtis White (Dow Corning, Midland, MI). It is a methanolic solution containing 42 wt % of this active ingredient. [Methyl-^{3}H]thymidine (20 Ci/mmol), [methyl-^{14}C]thymidine (58 mCi/mmol), and [2-^{3}H]mannose (25 Ci/mmol) were purchased from New England Nuclear (Boston, MA). Other chemicals were of reagent grade and were purchased from various commericial sources.

Preparation of Adsorbent

Dried alginate–magnetite beads (Fig. 1) were prepared by a method modified from that described by Burns et al.

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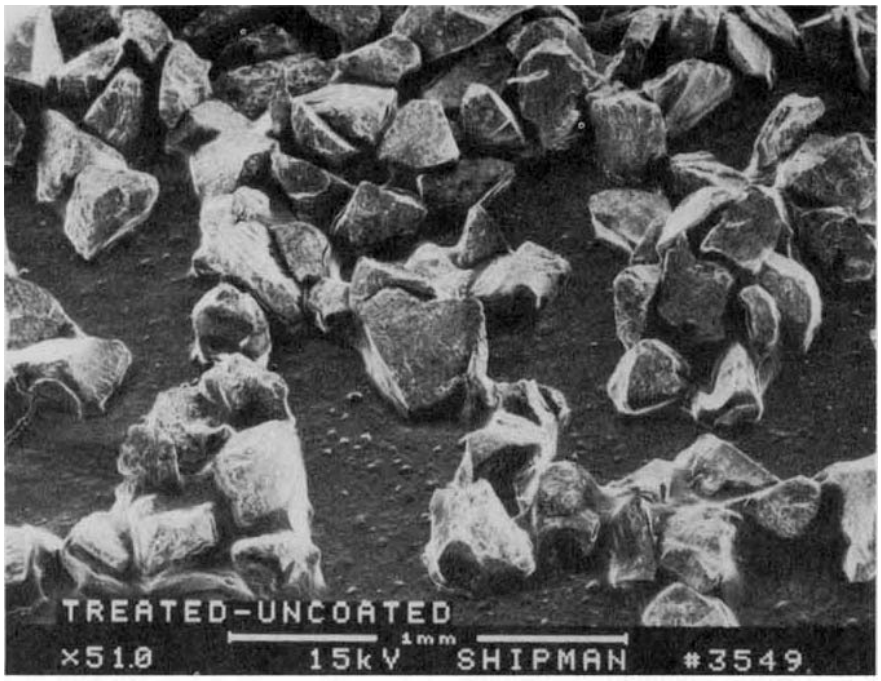

Figure 1. Scanning electron micrograph of the QAC-treated beads.

with the following modifications.[9] Barium chloride was used as a gel-inducing agent for better stability.[10] The beads were further stabilized by treating with glutaraldehyde in the presence of polyethyleneimine to avoid dissolution.[11] In brief, the alginate–magnetite gel spheres were suspended in 0.5% (w/v) polyethyleneimine–HCl in 50m*M* $CaCl_2$ (pH 7.0) for 24 h at room temperature with stirring. The gel spheres were washed briefly with H_2O and subsequently incubated in 1% (v/v) glutaraldehyde, 10m*M* sodium phosphate (pH 7.0) at room temperature for 1 min with stirring. To remove or "neutralize" the unreacted glutaraldehyde molecules, the gel spheres were washed 3 times with H_2O and incubated with 1*M* glycine (pH 7.0) for 30 min so that glutaraldehyde does not leach out from the beads. The treated preparation was washed in H_2O and stored at 4°C until the drying step. Dried beads with diameters between 0.15 and 0.25 mm were obtained by crushing the original spherical beads. A 1% solution of Si-QAC was prepared by diluting the stock solution in distilled water at pH 5. After the beads were added to the Si-QAC solution, the reaction temperature was raised to about 50°C for 10 min. The pH was adjusted to 10.5, and reaction continued for an additional 10 min. The beads were dried in an oven (100°C), rinsed several times with sterile deionized water (pH 7.0), and stored at 4°C.

Cell Culture

BSC-1 cells (an established line of African green monkey kidney cells) were grown as a monolayer in minimal essential medium (MEM) with Earle's salts supplemented with 10% fetal bovine serum (FBS) and 1.1 g/L sodium bicarbonate. Cells were passaged according to conventional procedures by using 0.05% trypsin plus 0.02% ethylenediaminetetraacetic acid (EDTA) in HEPES-buffered balanced saline (HBS).[12] Tissue culture flasks were incubated at 37°C in a humidified 3% CO_2–97% air atmosphere. Total cell counts were made using a Coulter counter equipped with a 100-μm orifice.

Virus and Plaque Assay

HSV-1 strain 148 was passaged in KB cells to prepare virus stocks of high titer (10^8-10^9 PFU/ml) as described previously.[13] HSV-1 was assayed by using monolayer cultures of BSC-1 cells grown in six-well cluster dishes. The cells were planted 3×10^5 cells/well in MEM(E) with 10% FBS and 1.1 g/L sodium bicarbonate. After 24 h the cell sheet was about 80% confluent and was inoculated with 0.2 mL virus suspension to be assayed and incubated for 1 h to permit viral adsorption. The cells were then overlaid with 3 mL medium containing 0.5% methocel (4000 cP) (Dow Chemical Midland, MI) incubated for another 2 days. After aspiration of the overlay, the cells were fixed and stained with crystal violet in 20% methanol, and the macroscopic plaques were enumerated.

Preparation of Labeled Virus

HSV-1 with [^{3}H]-labeled DNA was prepared using [^{3}H]thymidine by a method similar to the one described previously.[14] Monolayers of KB cells in 150-cm^2 tissue culture flasks were grown to 80% confluence and then infected with HSV-1 (multiplicity of infection, M.O.I. = 20) in virus growth medium [VGM, MEM(E) with 0.127 g/L L-arginine, buffered with 25m*M* HEPES, pH 7.4, at 22°C

and supplemented with 10% FBS]. Virus (M.O.I. = 0.1) was allowed to adsorb for 1 h at 37°C. Then each monolayer was rinsed twice with warm VGM without serum to remove unadsorbed viruses. Thirty milliliters of VGM was added to each flask for an additional 2 h incubation. The [^{3}H]thymidine was added to give a concentration of 17 μCi/mL in each flask, and the incubation was continued for 36 h to permit maximum production of supernatant virus. The radioactive suspension was then briefly sonicated and centrifuged at a low speed to remove cells and cell debris. The supernatant fluid was further centrifuged using a Beckman JA-21 rotor at 18,000*g* for 20 min at 4°C in a Beckman J-21 preparative centrifuge to pellet mature virions. The virus was resuspended in a small volume of medium [MEM(E) with 5% FBS], then further purified by sedimentation through 5–25% sucrose with a 45% sucrose cushion in a SW 50.1 rotor at 150,000*g* for 1 h in a Beckman L3-50 ultracentrifuge. The band of labeled virus was collected and dialyzed against HBS at 4°C. The virus was pelleted using a JA-21 rotor as described above and resuspended in MEM(E) with 5% FBS. The final preparations were stored at −76°C until retrieved. The double-labeled ([^{3}H] and [^{14}C]) HSV-1 was prepared by the same procedure described above except that [methyl-^{14}C]thymidine (10 μCi/mL) and [2-^{3}H]mannose (7 μCi/mL) were used to label viral nucleocapsid and envelope, respectively. The [2-^{3}H]mannose is commonly used as a specific viral envelope glycoprotein label as described by Eisenberg et al.[15] To facilitate the uptake of [2-^{3}H]mannose by the cells, glucose-free Dulbecco's modified Eagle's medium with 350 mg/L L-glutamine and 110 mg/L sodium pyruvate was used for the preparation of VGM.

Protein Assay

Samples collected in all experiments were cooled and stored at 4°C. The concentration of total protein in the solution was assayed by the method of Bradford.[16]

Batch Adsorption Experiments

During these experiments, adsorbents and viruses were continuously mixed in vials by a tube rocker (30 cycles/s) at room temperature. To prevent virus adsorption onto vial walls, the vials were filled with 5% BSA in HBS for 30 min and air dried after discarding the BSA solutions to coat the inner surfaces. Reaction mixtures of known composition were made by adding the stock solution to HBS at pH 7.0. A solution of 0.5% BSA in HBS was used in all experiments unless otherwise stated. All stock chemical solutions were filter sterilized and stored at 4°C.

In the equilibrium studies of virus adsorption, the time required to reach equilibrium was determined by periodically sampling over a 24-h period. In the kinetic studies, samples were withdrawn at predetermined time intervals and assayed for virus titers. Each adsorption experiment was performed at least twice and the averaged results were presented.

Mechanistic Study of Virus Inactivation

The experimental scheme in Figure 2 was designed to elucidate the mechanism of HSV-1 inactivation on a QAC-treated surface. The [^{3}H]-labeled HSV-1 was used in this experiment. The "control vial" contained the virus suspension without adsorbent while the "reaction vial" had 10 wt % QAC-treated beads. Both vials were shaken gently (30 cycles/s) at room temperature for 2 h. Then the first set of samples were collected from the supernatant of both vials and saved for the assays of infectivity and radioactivity. Sedimentation analysis also was carried out. Elution was subsequently performed at room temperature for 1 h by directly adding 4× strength eluant (1% tryptone, 10% FBS, and 1*M* glycine) to the suspension as described by Murray and Laband.[17] The second set of samples from both vials was collected and assays were performed accordingly. Although little variation was observed in replicate experiments, values were obtained in two trials and represent the means of four determinations.

Sedimentation Analysis of Labeled Virus

Changes in the sedimentation profiles of the radioactively labeled HSV-1 by the adsorption and elution steps were determined by rate-zonal centrifugation.[18] Virus suspension (0.4 mL) was layered onto a 5–45% sucrose gradient in HBS. Centrifugation was carried out using a SW 50.1 swing-out rotor at 150,000*g* for 45 min at 4°C in a Beckman model L3-50 ultracentrifuge, and fractions collected from the bottom of the gradient were analyzed for total radioactivity in scintillation cocktail. Counts per minute were determined in a Beckman LS 8100-Texas In-

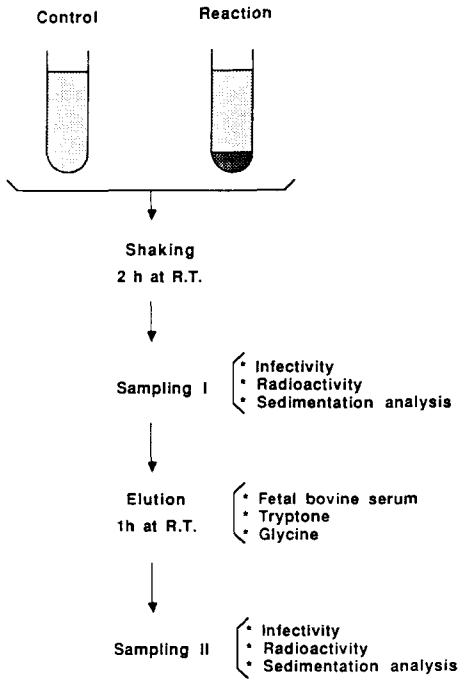

Figure 2. Experimental scheme for the mechanistic study of virus inactivation using QAC-treated beads.

struments 733 liquid scintillation spectrometer system. For samples with [^{3}H] and [^{14}C] double labeling, standard calibration was run to subtract [^{14}C] spillover radioactivity in [^{3}H] counting window.

RESULTS

Effect of Protein Concentration on HSV-1 Adsorption to QAC-Treated Beads

Using the experimental procedure described above, we showed the QAC-treated beads were able remove HSV-1 effectively even in the presence of 0.5% BSA by weight (Fig. 3). The virus titer, starting from 1.0×10^7 PFU/mL, dropped down more than 1 log in the first 5 min, was further reduced to 2.0×10^2 PFU/mL, then leveled off. The total titer reduction was close to 5 logs. On the other hand, the protein recovery was more than 90%. A parallel experiment using dried alginate–magnetite beads without QAC treatment showed that the titer reduction was only 8%. Hence, neither untreated beads nor thermal inactivation was the major cause of the observed 5-log titer reduction. In addition, the pH of adsorption medium (0.5% BSA in HBS) did not change throughout the experiment. As expected, BSA molecules compete with the viruses for the surface-binding sites. The binding capacity of the QAC-treated beads was decreased with increased BSA concentration (Fig. 4). The titer reduction was close to 5 logs for 0 and 0.5% BSA, 2 logs for 1% BSA, and only 1 log for 4% BSA. Presumably the rate of BSA binding to the surface is higher as the concentration is increased. These results suggest that the QAC-treated bead is a very effective virus adsorbent at low protein concentrations. However, the titer reduction at high protein concentrations is poor due to the competitive binding of protein molecules.

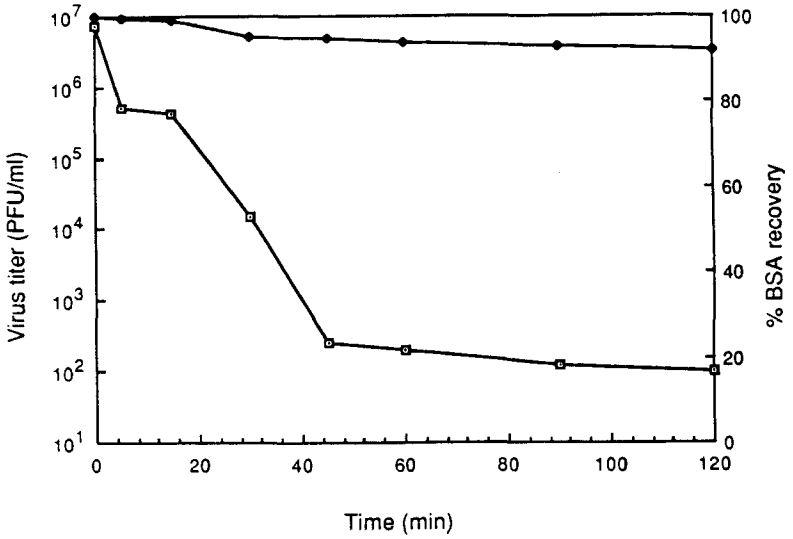

Figure 3. Effect of batch adsorption on HSV-1 titer reduction and BSA recovery using QAC-treated beads. Samples from the supernatant were assayed for virus titer (□) and BSA concentration (◆). The initial BSA concentration was 0.5%.

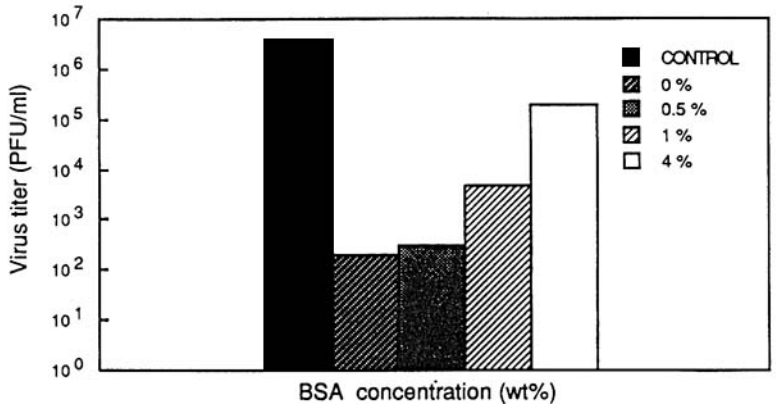

Figure 4. Effect of BSA concentration on HSV-1 titer reduction using QAC-treated bead. Control was the virus suspension containing no adsorbent. Batch adsorption experiments were carried out in solutions of various BSA concentrations for 2 h at room temperature with 10% QAC-treated bead.

Comparative Recovery of HSV-1 Infectivity and Radioactivity During Adsorption and Elution Steps

Data from sampling I and sampling II in Figure 2 were examined to compare the recovery of infectivity and radioactivity of the viruses in various stages of the experiment. Figure 5(a) shows the effects of adsorption and elution on the HSV-1 infectivity. The virus suspension was mixed with 10% by weight QAC-treated beads and gently shaken for 2 h at room temperature. The HSV-1 titer reduction was more than 4 logs. However, the elution experiments indicated that almost all the lost infectivity cannot be recovered. For the same set of samples, the effects of adsorption and elution on HSV-1 radioactivity are illustrated in Figure 5(b), which shows the results from samples of parallel experiments with 1:2 dilutions of initial virus titer. The radioactivity of the virus suspensions were consistently reduced by only 40% of the original value while the elution step recovered about half of the adsorbed radioactivity. In other words, the specific infectivity of virus in the suspensions was reduced drastically by the adsorption and elution steps.

Effects of Adsorption and Elution on Sedimentation Behaviors of Virus Suspensions

To elucidate the mechanism of enveloped virus inactivation on QAC-treated surface, double-labeled HSV-1 (with [^{3}H] in the envelope only and [^{14}C] in the nucleocapsid only) was used in adsorption–elution experiments. The sedimentation behaviors of the viral particles were examined by centrifugation through 5–45% sucrose gradient. Since it is a rate-zonal centrifugation, the resolution of particles is based mainly on size difference. Figure 6(a) shows the sedimentation profiles, both [^{3}H] and [^{14}C], of the virus suspension in which no adsorbent was added (control). There are two peaks appearing in the [^{14}C] profile of the control, whereas there is only one in the [^{3}H] profile. Notice that the first peak (from the bottom) of the [^{14}C] profile coincides with the only peak in the [^{3}H] profile. That is, evidently, the particles in the second peak of the [^{14}C] profile do not contain [^{3}H]-labeled viral particles. In addition, based on the fact that bigger particles settle faster than smaller ones as well as the infectivity assay results of each fraction [Fig. 6(a), inset], we determined that the viral particles in the first peak of the [^{14}C] profile are infectious virions of HSV-1 (180 nm diameter) while the ones in the second peak are noninfectious nucleocapsids of HSV-1 (105 nm diameter). The possible virus aggregates would stay in the leading region of the first peak (i.e., above 45% sucrose). Two points can be made by comparing the sedimentation profiles of the control [Fig. 6(a)] and that of the reaction [Fig. 6(b)]. First, the first peak of the [^{14}C] profile and the only peak of the [^{3}H] profile were almost totally eliminated. Second, the viral particles in the second peak of the [^{14}C] profile were enriched. These results suggest that nearly all the enveloped virus particles were adsorbed by the QAC-treated beads as indicated also

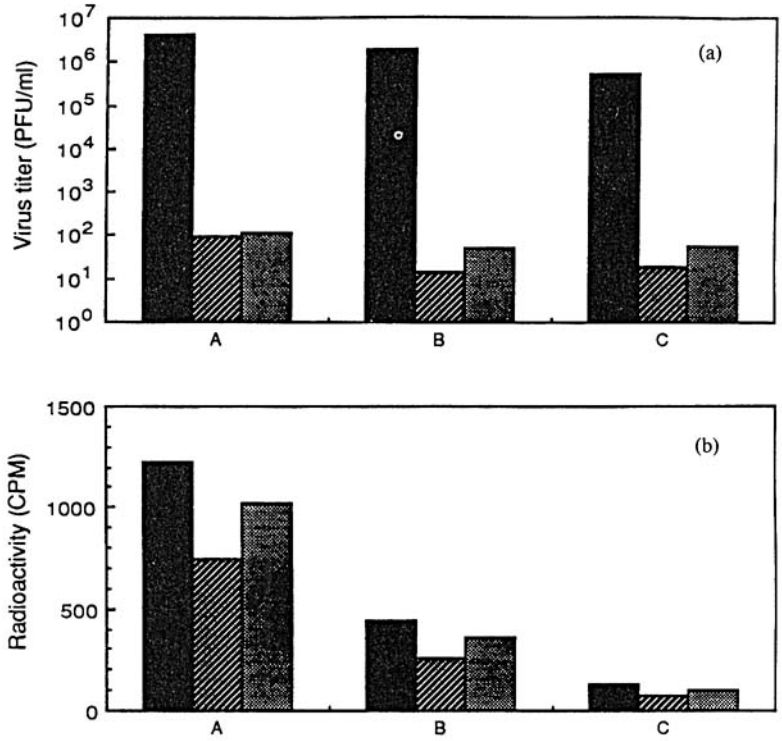

Figure 5. Comparative recovery of HSV-1 (a) infectivity and (b) radioactivity in various stages of the mechanistic study. A, B, and C were samples of parallel experiments with 1:2 dilution. The measurements of the control (■) that contains no adsorbent was compared with those of samples after adsorption (▨) and after elution (▩).

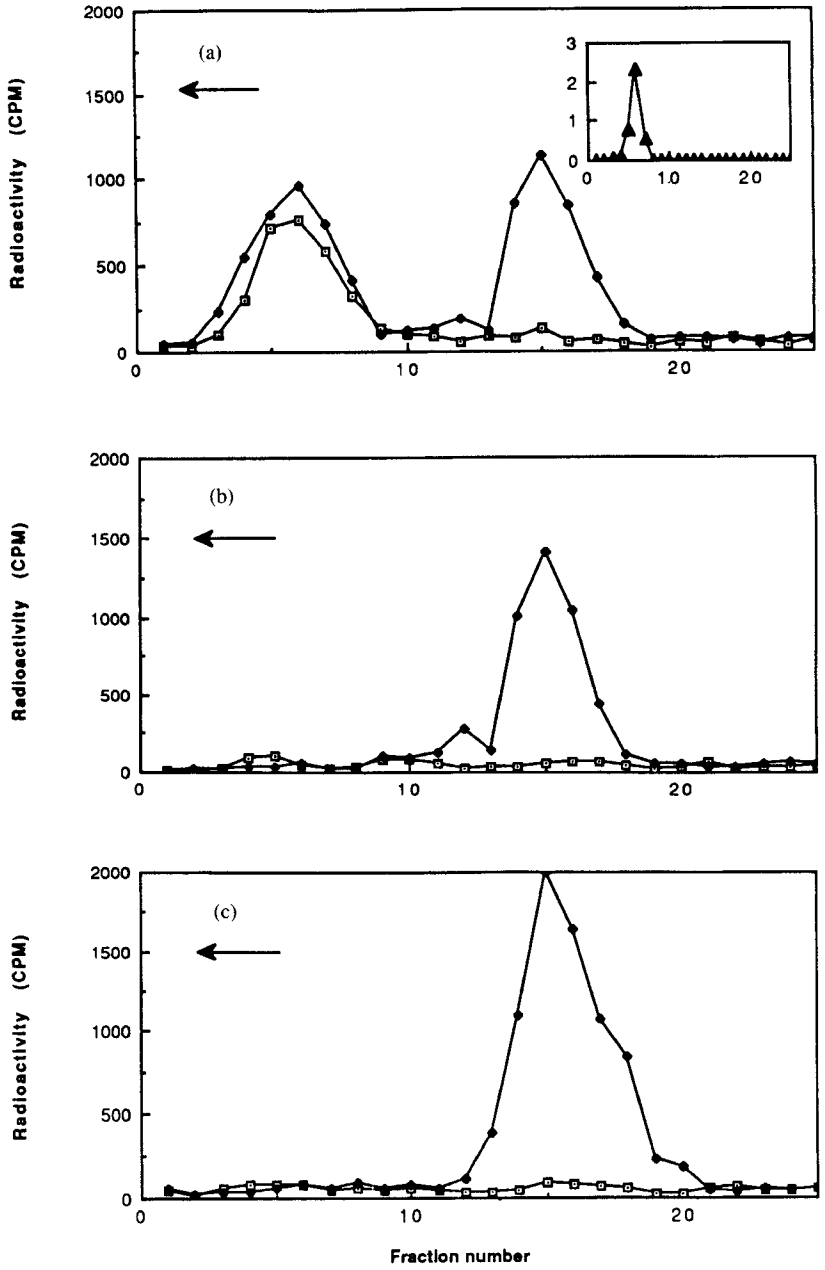

Figure 6. Effect of adsorption and elution on the sedimentation behaviors of double-labeled HSV-1 particles. Samples were analyzed by sedimentation in gradients (5% to 45% sucrose, SW 50.1, 150,000 × g, 45 min, 4°C). The arrows show the direction of sedimentation. The [^{14}C] (◆) and [^{3}H] (⊡) profiles in (a) are the control containing no adsorbent; the corresponding virus titer (PFU/ml × 10^7) in each fraction (▲) is shown in the inset. The sedimentation profiles of viral particles from sample I (after adsorption) and sample II (after elution) in Fig. 2 are shown in (b) and (c), respectively.

by the results of the infectivity assay [5-log titer reduction, as shown in Fig. 5(a)]. Furthermore, a portion of the [^{14}C] radioactivity carried by the viral DNA in the bulk solution can be attributed to the release of the nucleocapsids. The latter statement was proven by the elution experiment, which demonstrated the eluted viral particles were apparently noninfectious nucleocapsids [Fig. 6(c)]. We also analyzed the sedimentation profiles of the beads washed with HBS before the elution step. It was found that the profiles were very similar to Figure 6(c) with only one peak in the [^{14}C] profile at around fraction 15. The [^{3}H] radioactivities are very low in all fractions of Figures 6(b) and (c). This suggests that the envelope membrane of HSV-1 was tightly adsorbed by the QAC-treated surface, which is highly hydrophobic.

Infectivity of HSV-1 Adsorbed on QAC-Treated Surface

To investigate whether the virus particles were still infectious when they are adsorbed on the QAC-treated surface, we washed the beads taken out right after the

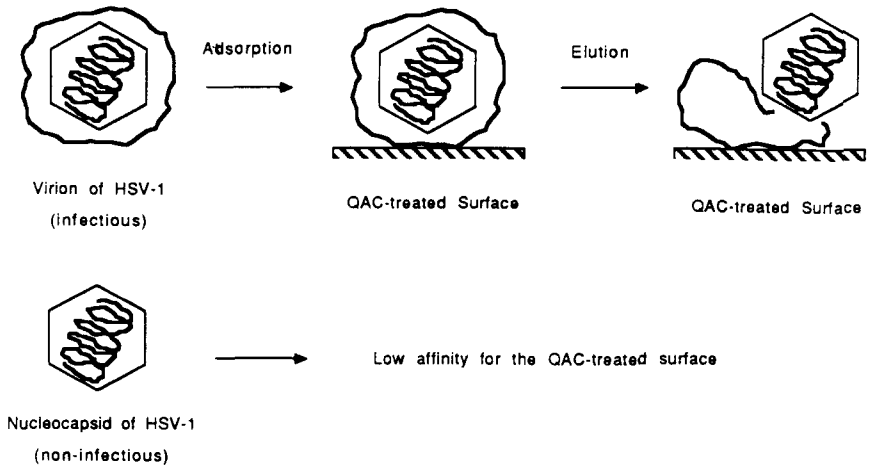

Figure 7. Proposed mechanism of HSV-1 inactivation on QAC-treated surface. The inactivation is caused by the disruption of the viral envelope with subsequent release of the nucleocapsid.

adsorption step and incubated them with a monolayer of BSC-1 cell. Plaques resulting from virus infection were formed surrounding all the beads tested. The plaques are not caused by the bead's movement as suggested by a control experiment using fresh QAC-treated beads without adsorbed viruses. However, it is possible that only a small fraction of the adsorbed viruses remain infectious.

DISCUSSION

These results demonstrate the ability of the QAC-treated surface to adsorb HSV-1, an enveloped virus, under various processing conditions. Hydrophobic and electrostatic interactions are the predominating forces which effect the adsorption of microorganisms to a QAC-treated surface.[3,5,6–8] For enveloped viruses, the hydrophobic interaction is presumably the major one since nonenveloped viruses show much lower affinity for the QAC-treated surface (unpublished data). The interactive effects of pH, ionic strength, and temperature on the virus adsorption are currently under study in our laboratory.

Based on the observation that virus retains its infectivity when adsorbed on a QAC-treated surface together with the results from infectivity and radioactivity assays as well as the sedimentation analysis, we were able to derive the following mechanism of inactivation of HSV-1 on the QAC-treated surface (Fig. 7). The mechanistic study indicates that HSV-1 was inactivated by the QAC-treated surface by virtue of the disruption of the viral envelope. This damage leads to an irreversible loss of infectivity. None of the evidence indicates that the released nucleocapsids are further disrupted. The virions of HSV-1 are readily adsorbed by the QAC-treated surface due to strong hydrophobic and other interactions. For the adsorbed virions, the part of envelope that is not in contact with the QAC-treated surface is still intact. The nucleocapsid can penetrate into a host cell by fusing the intact part of the envelope with the cell plasma membrane. The viral envelopes are disrupted when the elution is employed and hence only the nucleocapsids are released. The nucleocapsid shows a much lower affinity for the QAC-treated surface.

The QAC-treated bead made of dried alginate and magnetite used in this study is nonporous. To remove the viral contaminants from a protein solution, adsorbents of a nonporous nature are preferred in order to eliminate nonspecific adsorptions of desired proteins onto an intraporous surface.

Despite the fact that the diffusion coefficient of BSA is one order of magnitude higher than that of HSV-1, the adsorption rate of HSV-1 onto the QAC-treated surface is greater than that of the BSA. This result indicates that surface reaction, instead of diffusion, is the controlling step of the binding process. HSV-1 can readily bind to the QAC-treated surface simply because the viral envelope is hydrophobic in nature. The kinetic study indicates that HSV-1 was adsorbed/inactivated rapidly in the first hour following approximately a first-order reaction kinetics with respect to the remaining virus concentration in the solution. The leveling off of titer reduction is probably due to the effect of steric hindrance from previously adsorbed viruses and proteins. It has been shown that the residual virus titer can be completely eliminated by adding additional fresh beads to the suspension.

The significant reduction of virus adsorption capacity in solutions with high BSA concentration (more than 1%) reflects the difficulty involved in the use of the QAC-treated beads to remove viruses from solutions with high protein contents such as plasma and serum. In this case, the use of affinity adsorbents based on specific interactions such as antibodies or cellular receptors appears to be more appropriate. Even so, QAC-treated beads certainly can find wide applications in treating blood products that have low protein content in solution, such as packed red blood cell and platelet concentrate. In order to avoid the possible damage caused by direct contact, however, a porous membrane barrier that allows free passage of viruses should be used to exclude the entry of cells.

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Surface Kinetic Test Method for Determining Rate of Kill by an Antimicrobial Solid

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An antimicrobial-surface kinetic test which maximizes probability of cell-to-surface contact has been developed. The measurement of rate of kill by a nonleaching antimicrobial surface is based on the number of surviving bacterial cells at specific times of exposure to various amounts of total treated surface area of test substrate. This method gives information for direct comparison of rate of kill for a variety of antimicrobial surfaces in terms of rate of kill per square centimeter of surface area. Data obtained by this method can also give valuable dose response application information as an indication of the exponential efficiency of concentration in terms of treated surface area.

Recent publications from this laboratory (4, 13) described the creation of durable antimicrobial surfaces by the application of a cationic alkoxysilane, 3-(trimethoxysilyl)propyldimethyloctadecyl ammonium chloride, to a spectrum of surfaces. In contrast to other antimicrobial agents for which durable residual-surface treatments have been claimed (1, 8, 12), the activity of the organosilicon-treated surface was not ascribed to slow-release, solution-active chemicals. Both radioisotope and bioassay procedures demonstrated that the agent was not released from the surface.

Standard methods for determining bactericidal activity of antimicrobial agents in terms of rate of kill per concentration of compound in solution are well established (2). Similar methods for determining dose response relationships for active surfaces are not. Because of the apparent catalytic nature of substrate treated with the cationic alkoxysilane, i.e., because there is no consumption of the chemical during microbial kill, a procedure used to measure kinetics of chemical catalysts was modified in an attempt to obtain a sensitive method for determining rate of reaction and obtaining dose response curves for biologically active material. This communication describes the development and use of the method.

MATERIALS AND METHODS

Preparation of resting-cell suspension. *Escherichia coli* B (ATCC #23226) was harvested from the logarithmic phase of growth in nutrient broth by centrifugation at 15,000 rpm for 15 min in a refrigerated Sorval RC2-B. Harvested cells were suspended in sterile physiological saline and washed three times by centrifugation. The washed cell pellet was suspended in fresh sterile saline and allowed to equilibrate at room temperature for 30 min to deplete endogenous metabolites. The resultant resting-cell suspension was diluted further in sterile saline to the desired cell concentration. Optical density and pour plate techniques were employed to measure concentration of cells.

Preparation of treated surface. Antimicrobial-surface test material was prepared by treatment of a type of commercial silica (Min-u-sil; Pennsylvania Glass Sand Corporation) which has a 10μ mean particle size and contains a surface area of 11.0 cm^2/mg with alkyl chain ^{14}C-labeled $(CH_3O)_3Si(CH_2)_3{}^+N(CH_3)_2C_{18}H_{37}Cl^-$ to a level of seven molecular layers. Surfaces were washed by centrifugation, and the supernatant fluid was assayed for soluble, unbonded alkoxysilane. At the level of treatment used, no unbound material was detected by either ^{14}C analysis or bioassay procedures in the decanted supernatants. By this procedure, ^{14}C analysis of the treated Min-u-sil showed 11.62×10^{-10} mol of organosilane per mg of sample.

Test procedure. The "surface kinetic test" was performed by the precise addition of 100 cm^2 of treated Min-u-sil to 2 ml of *E. coli* B resting-cell suspension in a screw-cap tissue culture tube (15 by 125 mm). The contents of the inoculated and sealed tube were blended in a Vortex mixer for 5 s, placed at 37°C, on a Fisher Roto Rack, and rotated through 360° at 29 rpm. Individual tubes were removed at specific time intervals, diluted to a concentration of 20 to 200 cells per ml in Letheen Broth (Difco Laboratories) to inactivate the cationic treatment, and pour plated in Letheen agar. Viable cell counts were made for each sampling time in triplicate after incubation for 24 h at 37°C. Inoculum control tubes consisting of resting cell suspensions, as well as inoculated, untreated Min-u-sil control tubes, were included in each experiment.

RESULTS

By the test procedure described above, solid

surfaces prepared by immobilization of 3-(trimethoxysilyl)propyldimethyloctadecyl ammonium chloride on Min-u-sil were found to be active against resting-state *E. coli* B cells (Table 1). Within 15 min, a 99% reduction in viable bacterial count was achieved in cells exposed to 50 cm^2 of active surface per ml suspended in sterile saline. When a standardized inoculum of between 1,000 and 8,000 cells is used, the rate of kill ($-k$) appears to be a first-order rate process commonly used to quantitate the exponential death of microorganisms (5). This process is described by the equation $S_t = S_0e - kt$, where S_t = surviving cells at time (t), S_0 = initial number of organisms at zero time, and $-k$ = rate of exponential death when $\log_e S_t$ is plotted against time. In Fig. 1, the surface activity rate can be described as $-k = [(\log_e S_t - \log_e S_0)/\text{time}] = 0.283$. Therefore, $\log_e$ reduction per square centimeter per minute = 0.283/50 = 0.0057.

The surface activity rate of kill ($-k$) of Min-u-sil treated with 3-(trimethoxysilyl)propyldimethyloctadecyl ammonium chloride was found to be a function of treated surface area, with $\log_e$ bacterial cell reduction per minute increasing with increasing surface area (Table 2). By using a constant initial inoculum level (S_0), one obtains a graded concentration effect of total treated surface area (Fig. 2; Table 3).

Therefore, a statistically significant ($P < 0.05$) response (determined by Student's t test) which correlated well at each concentration level at similar times of exposure was achieved.

The relationship of response time to area of antimicrobial surface (dose) was determined from the data in Table 2. The time (t) to kill 50% of the initial number of viable cells was calculated for each surface area (A). A sigmoid curve is obtained (Fig. 3) when $\log_t$ is plotted against $\log_A$ indicating that the change in exponential effectiveness below 25 and above 100 cm^2/ml decreases significantly at this inoculum level. The regression line in the linear portion of the curve results in a rate of decrease (x) in response time with increasing surface area of 0.781. $x = (\log_{t-a})/\log A$, where $a = \log_t$ at zero surface area.

In the linear portion of the curve, $A_2{}^x t_2 = A_3{}^x t_3 = A_4{}^x t_4 = 36.4$. Therefore, ${}_{25}\int^{100} A^x t = K$ (con-

TABLE 1. *Survival of E. coli in the presence of active Min-u-sil surfaces*

Time (min)	Viable cells/ml			% Reduction
	Control[a]	Control[b]	Expt[c]	
0	3,750	3,750	3,750	0
5	4,370	3,900	1,600	63
15	5,000	3,700	29	99
30	4,200	4,300	1	99.9
45	4,100	3,900	0	>99.9
60	3,500	4,400	0	>99.9
75	4,200	4,400	0	>99.9
90	3,600	4,900	0	>99.9
120	3,600	4,600	0	>99.9
24 h	5,250	6,000	0	>99.9

[a] Inoculum control in 2 ml of physiological saline.
[b] Untreated control: 10μ Min-u-sil, 50 cm^2/ml.
[c] Active surface: 10μ Min-u-sil, 50 cm^2/ml, treated with 3-(trimethoxysilyl)propyldimethyloctadecyl ammonium chloride.

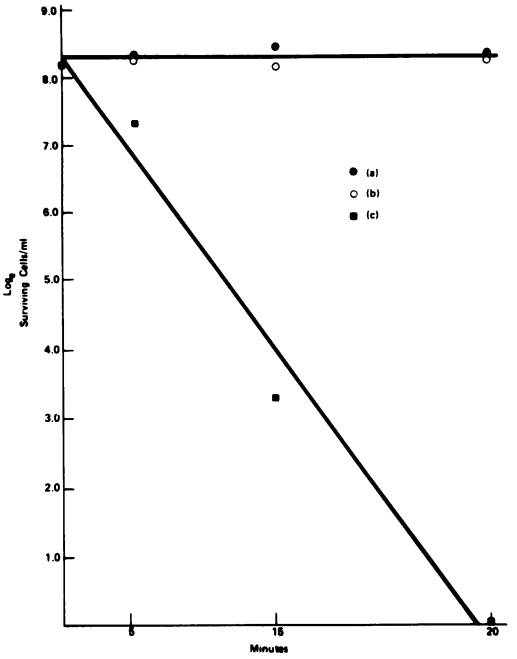

FIG. 1. *Rate of kill of E. coli B in the presence of: (a) physiological saline, (b) 10μ Min-u-sil, 50 cm^2/ml, or (c) 10μ Min-u-sil, 50 cm^2/ml, treated with 3-(trimethoxysilyl)propyldimethyloctadecyl ammonium chloride.*

TABLE 2. *Rate of kill of E. coli B with time for various concentrations of antimicrobial surface*[a]

Surface area (cm^2/ml)		Response time (min)[b]	Log response time	($-k$) $\log_e$ reduction/min
A	$\log_A$			
12.5	1.0969	5.924	0.773	0.117
25.0	1.3779	5.163	0.713	0.133
50.0	1.6990	3.487	0.542	0.199
100.0	2.0000	1.751	0.243	0.396
400.0	2.6021	1.642	0.215	0.423

[a] Min-u-sil (particle size, 10μ) treated with 3-(trimethoxysilyl)propyldimethyloctadecyl ammonium chloride. Response time was defined as time required to reduce inoculum (10,000 cells per ml) by 50%.

stant). The surface area (dose) represents 1.66 $\times 10^{-11}$ mol/cm^2 per monolayer. Doubling of the surface area (dose) increases the antimicrobial effectiveness by a factor of 1.718.

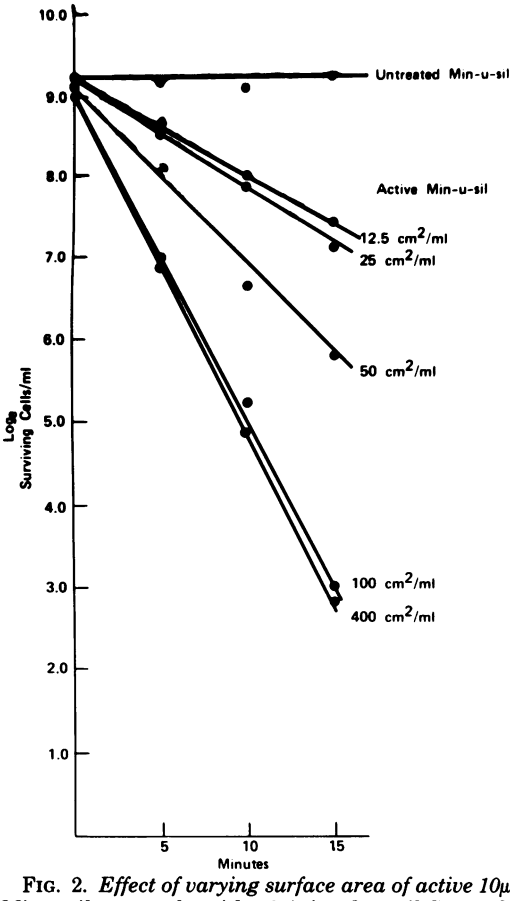

FIG. 2. *Effect of varying surface area of active 10μ Min-u-sil treated with 3-(trimethoxysilyl)propyldimethyloctadecyl ammonium chloride on the rate of reduction of viable E. coli B.*

The expected non-linearity at low concentrations, typical of dose response curves, was not due to tolerance. Positive growth was obtained after subculturing exposed bacterial cells to fresh growth media. Isolates from this culture were transferred three consecutive times and retested against freshly treated Min-u-sil. Activity against this isolate did not significantly differ from that in the initial test; therefore, the decreased rate ($-k$) of less than 25 cm^2/ml cannot be attributed to the development of resistance by *E. coli* B.

The decreased rate of kill could not be attributed to loss of treatment from the Min-u-sil. In separate experiments, Min-u-sil treated to the same level with ^{14}C-labeled 3-(trimethoxysilyl)propyldimethyloctadecyl ammonium chloride showed no loss of radioactivity to solution after extensive mixing (up to 24 h) in the surface kinetic test.

The response rate in Fig. 3, determined for increased amounts of Min-u-sil to confirm the function of limiting surface area per rate of kill, showed very little potentiation of activity above 100 cm^2/ml at this inoculum level.

The ratio of viable to nonviable cells may alter adsorption-desorption kinetics and thus affect probability of contact with active sites as found in studies of conventional solution-active agents (6, 9). Additional tests at varying concentrations

TABLE 3. *Graded concentration effect of surface area*

Surface area (cm^2/ml)	$-k$	Correlation coefficient	Student's t
12.5	0.117	0.9485	2.9934
25.0	0.133	0.9243	15.978
50.0	0.199	0.9984	25.2199
100.0	0.396	0.9986	27.0036
400.0	0.423	0.9596	4.8247

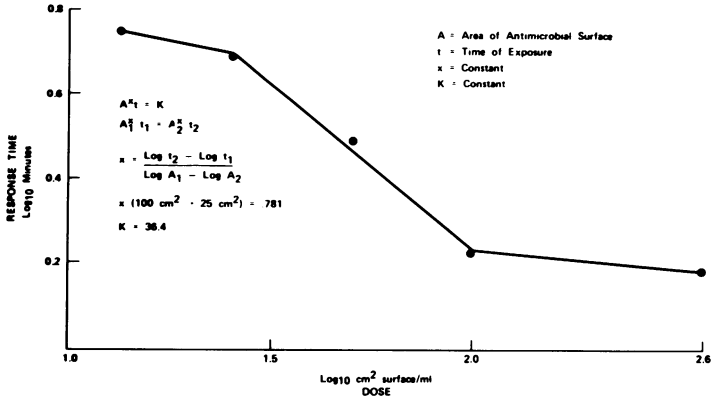

FIG. 3. *Dose response of 10μ Min-u-sil treated with 3-(trimethyoxysilyl)propyldimethyloctadecyl ammonium chloride.*

of initial bacterial challenge level (Table 4) showed that $-k$ is affected by total bacterial cells per milliliter per square centimeter of surface area. At a constant surface area of treated Min-u-sil (50 cm^2/ml), the rate of kill ($-k$) decreases with increasing concentration of initial bacterial inoculum. A preliminary standard curve (Fig. 4) was obtained from these data, indicating that the relationship can be described as the slope (m) when log S_0 (cells per ml at zero time) is plotted against $-k$ (rate of kill). In this range of S_0 (1,000 to 8,000 cells per ml), $m = -0.286$ and $t = 30.194$. A significant dependence of rate of kill on S_0 is observed in this initial inoculum range ($P < 0.05$). A standard treated surface of known activity should be included with each test of unknown antimicrobial surface to confirm this relationship. Additional tests at S_0 above 10^5 cells per ml showed very slight dependence of $-k$ on S_0. However, the sensitivity of the method was greatly reduced with a subsequent increase in experimental error. All of our rate calculations have been derived from the portion of the test (between 1,000 and 8,000 cells per ml) which fits first-order kinetics. However, one must recognize that the effects of cell population noted above decree that the overall process must be described as second order.

TABLE 4. *Effect of initial inoculum level (S_0) on the rate of kill ($-k$) of E. coli B at a constant concentration of active surface area*[a]

Surface area (cm^2/ml)	S_0 (cells/ml)	Log$_e$ S_0	($-k$) (Log$_e$ reduction/min)
50	1,321	7.1861	0.437
50	1,459	7.2855	0.413
50	3,988	8.2911	0.285
50	8,181	9.0096	0.208
50	8,243	9.0171	0.202

[a] Chemical used was 3-(trimethoxysilyl)propyldimethyloctadecyl ammonium chloride-treated 10μ Min-u-sil. See text for explanation of variables.

DISCUSSION

The organosilicon compound 3-(trimethoxysilyl)propyldimethyloctadecyl ammonium chloride is capable of creating highly active antimicrobial surfaces when durably affixed to a variety of materials. Although the activity of these solid surfaces can be described in terms of quantal data, it should be recognized that the expression of death rates of microorganisms, which are exponential in character, cannot be adequately described in terms of percentage (10).

The quantitative rate of kill observed when antimicrobial surfaces were incubated with resting-cell suspensions of *E. coli* B in a sealed rotating tube was found to be an exponential function when log$_e S_t$ was plotted against time.

At a constant level of initial inoculum (S_0) and at various concentrations of antimicrobial surface area (in square centimeters per milliliter) and times of bacterial cell exposure (t), the rate of kill ($-k$) increases with increasing surface area.

A preliminary dose response curve was obtained for antimicrobial solid surfaces relating exponential effectiveness of the concentration of active material in terms of square centimeters of surface area.

The surface kinetic test method we have developed allows quantitative reproducible measurement of the activity of antimicrobial solid surfaces in terms of the well-defined kinetics already established for measurement of solution-active agents. To determine the dose response relationship by this method, one substitutes

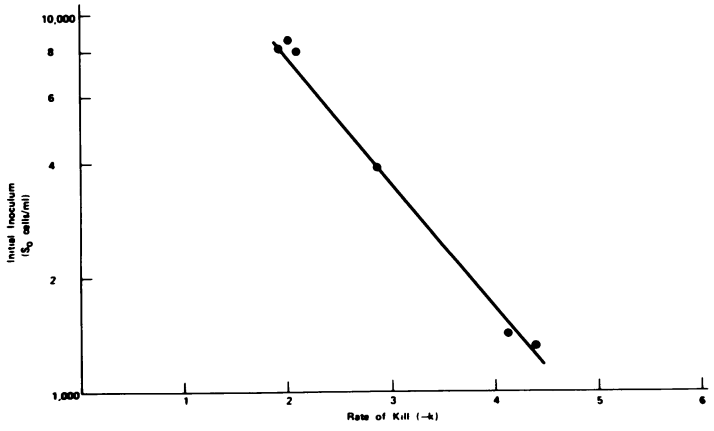

FIG. 4. *Standard curve for rate of kill of 10μ Min-u-sil (50 cm^2/ml) treated with 3-(trimethoxysilyl)-propyldimethyloctadecyl ammonium chloride in most sensitive range of E. coli B initial inoculum level.*

square centimeters of active surface area for parts per million or micrograms of drug per milliliter.

Although the idea of measuring the efficacy of solution-active agents on surfaces is not new (11), the quantitative measurement of rate of kill by solid surfaces which themselves serve as the active agent is new. If, as expressed by Klarmann et al. (7), the ideal method of control of microorganisms should involve creation on the exposed material surfaces of a persistent antibacterial potential (considerable effort has been expended on the development of such surfaces [3]), methods for the accurate assessment of this activity are needed. The surface kinetic test method described above measures this antimicrobial potential.

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In Vivo Study of an Antimicrobial Surgical Drape System

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We performed a double-blind clinical study to determine the efficacy of nonwoven laparotomy drapes in which 3-(trimethoxysilyl)propyldimethyloctadecyl ammonium chloride, an antimicrobial agent, was chemically bonded to the absorbent reinforcement surrounding the fenestration. The reinforcement portion of the surgical drape that contained the fenestration was segmented into four identical-appearing sections, two on each side of the fenestration. One segment on each side was antimicrobial. The locations of the treated segments were randomly varied. At the end of each operation, test strips were removed. Bacteria were harvested from each segment by mechanical agitation. Bacterial CFU were counted. There were 110 surgical cases in the study, including clean, clean contaminated, and contaminated procedures. Data analysis divided the cases into two distinct groups. Group 1 was composed of 59 cases in which less than 30 total CFU was recovered from the four test samples. The average duration of surgery for this group was 1.8 h. Group 2 was composed of 51 cases in which bacterial recovery was in excess of 30 CFU per procedure (range, 30 to 25,000 bacterial CFU). The average duration of surgery was 3.3 h. Bacterial reduction in the treated strips was 84%. The most common organisms identified on the laparotomy drapes were *Staphylococcus epidermidis*, *S. hominis*, and *Micrococcus luteus*. This study demonstrated that the reinforcement of a laparotomy drape is a reservoir for potential pathogens. It demonstrated that an organosilicon quaternary ammonium antimicrobial agent covalently bonded to the reinforcement reduced the number of potential pathogens surrounding the surgical incision by 84%, independent of the size of the bacterial challenge.

It has been estimated that 30,000 to 60,000 organisms are deposited on a 3- to 4-m^2 sterile field during every hour of major operations. In a recent 2-year study of 15,207 patients admitted to a hospital, there were 1,851 nosocomial infections reported, for an infection rate of 12.8%. Postoperative wound infections were the most common nosocomial infections encountered in the surgical services during this study. They accounted for one-third to one-half of all of the infections in the patients studied by Egoz and Michaeli (4). It has been found that the surgical wound infection rate increases from 1% for operations lasting 30 min to 14% for operations lasting 3.5 h (8).

One of the primary sources of bacterial contamination of wounds during surgery has been operative personnel. Charnley and Eftekhar (2) have shown that bacteria from a surgeon's skin penetrate clean scrub suits and sterile gowns to reach the sterile field. However, difficulty has arisen in trying to document that the organisms generated by the personnel in the operating room are the primary cause of wound infections. In a computer analysis of factors influencing surgical wound infection, Davidson et al. (3) cited the degree of contamination of the wound with microorganisms to be the most important determinant in the development of perioperative infections.

The preferred use of nonwoven barriers for the surgical staff and patient has been well documented (1, 6, 7, 12, 13, 16). Now nonwoven drapes have been developed with a broad-spectrum organosilicon quaternary ammonium antimicrobial agent covalently bonded to the absorbent reinforcement that surrounds the fenestration. This bactericidal fabric should reduce the number of viable bacteria on the surface of the drape. In vitro data have demonstrated this antimicrobial agent to be effective against *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli*, *Salmonella typhi*, *Mycobacterium tuberculosis*, *Pseudomonas aeruginosa*, *Enterobacter aerogenes*, *Candida albicans*, several *Asperigillus* species, *Trichophyton* species, and other potential pathogens (5, 10, 11). Furthermore, the antimicrobial fabric has been shown in the laboratory to be effective against the same series of potential pathogens. The antimicrobial fabric is capable of reducing the number of bacterial CFU recoverable from the fabric by 91% within 15 to 30 min when compared with a nonantimicrobial control fabric (5) (C. Herring, personal communication). The purpose of the present work was to establish the efficacy of the drapes by means of a clinical study and demonstrate that an antimicrobial draping system can reduce the number of potential pathogens surrounding a surgical incision.

MATERIALS AND METHODS

All of the surgical procedures were performed by the same surgeon in the surgical suites normally used by his service. Clean, clean contaminated, and contaminated surgical procedures were included in the study. All of the procedures allowed appropriate usage of the modified laparotomy drape developed for the study. The surgical cases included in the study varied in length from 0.5 to 6 h. The surgical team wore nonwoven masks, hair covers, and shoe covers. All other wearing apparel and fabrics used on the patient or by the surgical team were closely woven, washed linen.

Preoperative patient preparation included washing the wound site with a standard iodophor scrub solution followed by a standard iodophor prep solution. After the iodophor solution had dried, the special laparotomy drapes were

* Corresponding author.

† Julius Conn died during the preparation of this report. We dedicate this small token of our combined efforts to his memory.

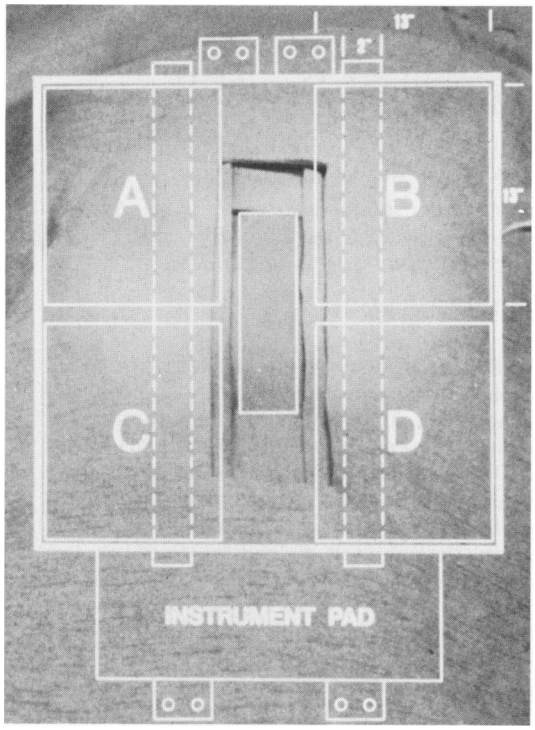

FIG. 1. A standard laparotomy drape reinforcement was modified to consist of four sections (A, B, C, and D), and only two were made of antimicrobial fabric. The locations of the antimicrobial sections were randomly varied. The standard test sections are shown by dotted lines.

placed in the usual manner. The special fenestrated laparotomy drape was the only variable from the routine prepping and draping of the surgical team. (The fenestration is the opening or hole in a surgical drape through which surgery is performed.)

To ensure unbiased sampling, special nonwoven, fenestrated drapes were manufactured for this study by using good manufacturing practices as required by the U.S. Food and Drug Administration. The experimental drapes were standard nonwoven, nonantimicrobial laparotomy drapes on which four 13- by 13-in. (1 in. = 2.54 cm) swatches (A, B, C, and D) of identical-appearing fabric had been attached on the reinforced area surrounding the fenestration (Fig. 1). Two of the swatches were treated with an antimicrobial agent, and two were untreated. Each drape was given a code number, and the locations of the antimicrobial swatches were recorded during the manufacturing process. The positions of the treated and untreated swatches were not known to anyone associated with the study. The positions of the swatches were randomized at the time of manufacturing. The study was conducted by a double-blind protocol. The antimicrobial agent covalently bonded to the treated swatches was 3-(trimethoxysily)propyldimethyloctadecyl ammonium chloride, as used in in vitro studies (5, 10, 11).

At the end of each surgical procedure, standardized 2- by 13-in. patches of swatches A, B, C, and D were aseptically removed from the drape with a clean scalpel and a sterile measuring template. These patches were placed in labeled, sterile, disposable petri dishes. The drape specimens were taken to the microbiology laboratory for immediate processing.

Within 30 min after the operation was completed, each patch was placed into a 250-ml sterile disposable flask containing 75 ml of letheen broth (Difco Laboratories, Detroit, Mich.) adjusted to pH 9.5 with NaOH. Control studies with letheen broth adjusted to pH 7.2 determined that the higher-pH broth did not affect the bacterial survival rate when exposure time was limited as described above. This broth is an accepted neutralizer of the bactericidal activity of quaternary ammonium compounds. The flask was placed on a wrist action shaker and agitated at the highest setting for 15 min. After agitation, the letheen broth was decanted from the flask and filtered through a sterile 0.22-μm (pore size) microporous filter. The filter was then removed and placed on a nutrient pad (Sartorius) in a 50-mm (diameter) petri dish. In some instances, when it was apparent that the letheen broth was highly contaminated, samples of the broth were filtered and counted. This was done to prevent clogging of the filter. The nutrient pad was rehydrated with sterile deionized water containing 1.0% yeast extract. The microbiological specimens were then placed in a humidified incubator at 36°C. The bacterial CFU on the microporous filters were counted and photographed after 72 h of incubation.

Identification of the bacterial isolates was done by standard clinical microbiological techniques. Minitek Enterobacteriaceae II (BBL Microbiology Systems, Cockeysville, Md.), the Staph-Ident system (Analytab Products, Plainview, N.Y.), Sero-STAT Stap (Scott Laboratories, Inc., Fiskeville, R.I.), and the Minitek aerobic gram-positive cocci test (BBL) were used as directed by the manufacturers.

RESULTS

Scanning electron micrographs. To test the antimicrobial characteristics of the treated and untreated fabrics used in this study, we obtained electron micrographs of the fabrics incubated with *E. coli*. These scanning electron micrographs showed that the morphology of bacteria was greatly altered after 15 min of contact with the antimicrobial-agent-treated fabric (Fig. 2B). The same organisms in contact with untreated fabric remained unchanged for at least 2 h (Fig. 2A). The obvious change in bacterial morphology attributed to the antimicrobial fabric is evidence that the bacterial cell wall membrane complex has been disrupted as postulated by Hugo (9) as the mode of action for this class of antimicrobials agent and agrees with the work of Malek and Speier (J. Coated Fabrics **12**:38-45, 1982) and Richards and Cavill (14).

Surgical procedures. The experimental drape used in this study was a modified, fenestrated, nonwoven laparotomy drape. Therefore, the majority of the procedures involved abdominal incisions. The surgical procedures by general type were as follows: vascular, 35%; liver and biliary tract, 12%; gastrointestinal (including resections, ostomy, etc.), 10%; hernia repair, 9%; miscellaneous (debridement, biopsies, abscess drainage, mastectomies), 34%.

Bacterial isolation. One hundred and ten surgical procedures were analyzed during this study. Analysis showed that the bacterial CFU recovered from the drapes divided the surgical procedures into two distinct groups. The groups were determined by the total number of CFU isolated from a single set of drape samples.

In group 1, the bacterial CFU recovered from each case totaled less than 30. Analysis of this group indicated that a comparison of the number of organisms recovered from the antimicrobial portion of the drape versus the CFU recovered from the nonantimicrobial drapes was not statistically relevant. This group was composed of 59 drapes in which the

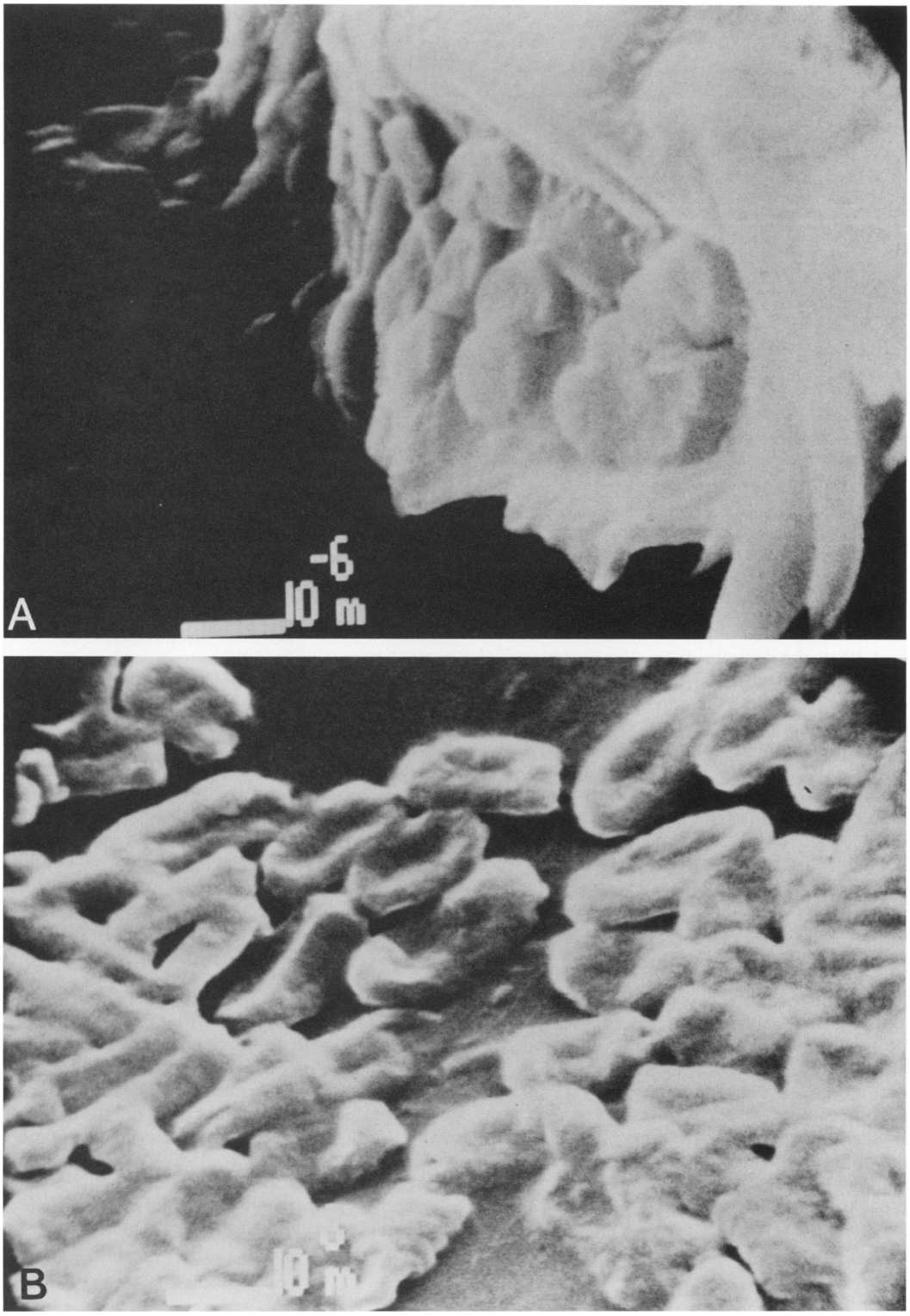

FIG. 2. Scanning electron micrographs of *E. coli* exposed to untreated and antimicrobial-agent-treated fabric (magnification, ×12,000). *E. coli* was suspended in phosphate-buffered water, and portions were placed on appropriate fiber samples. The specimens were incubated at 20°C for 120 min for the control (A) and for 15 min for the antimicrobial sample (B) in a humidified chamber. After incubation, the samples were rapidly vacuum dried and coated with gold. The samples were then examined and photographed with a Cambridge SEM-Stereoscan Mark II. Note the depressed centers of the bacteria on the treated fabric (B) compared with the bacteria on the untreated fabric.

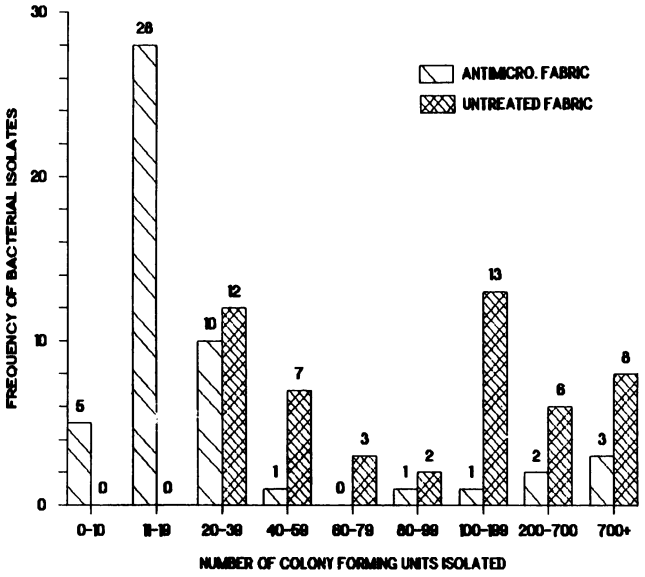

FIG. 3. Distribution frequency of the bacterial isolates recovered from the antimicrobial fabric and untreated fabric swatches from group 2. The actual number of surgical cases in which the indicated number of bacterial isolates recovered from the antimicrobial or untreated swatches is given at the top of each column.

mean number of bacterial isolates from the antimicrobial swatches was 4.5 CFU with a median of 1.9. The nonantimicrobial swatches had a mean bacterial recovery of 7 CFU with a median of 3.1. The range in total CFU was 1 to 29, and the mean length of surgery was 1.8 h with a median of 1.5 h.

Group 2 consists of 51 cases in which more than 30 CFU was isolated. The mean CFU for the antimicrobial swatches was 184 versus 1,172 CFU for the nonantimicrobial swatches. The mean duration of surgery was 3.3 h with a median of 2.9 h. Figure 3 demonstrates the frequency distribution of the bacterial isolates from the antimicrobial and nonantimicrobial swatches. Table 1 lists the numbers of CFU recovered from various locations on the surgical drapes included in group 2.

When each surgical procedure was individually analyzed for bacterial reduction, the bacterial reduction ranged between 15 and 99.9%. The average bacterial reduction percentage was 84.4%. Figure 4 graphically illustrates the bacterial reduction percentage frequency of the surgical procedures in group 2.

Analysis of the actual bacterial recoveries given in Table 1 showed that the data had positive skewness. The skewness is attributable to the clean contaminated and contaminated cases in which exceptionally large numbers of bacteria were isolated (greater than 1,000 CFU).

The surgical procedures from which the greatest number of isolates were recovered all demonstrated high bacterial reduction rates attributable to the antimicrobial fabric. In actuality, the average bacterial reduction percentage for this subgroup of cases was 83%, and the bacterial reduction percentage for the subgroup in which the bacterial isolates were less than 1,000 was 88%.

Bacterial identification was performed on the isolates from 64 cases. Since the organisms killed by the antimicrobial fabric could not be determined, analysis of the percentage of cases from which a particular organism was isolated was performed. Table 2 lists the organisms isolated and identified and the percentage of cases in which that particular bacterium was identified. *S. epidermidis*, *S. hominis*, and *Micrococcus luteus* were the most commonly isolated organisms.

DISCUSSION

The standard laparotomy drape used in this study had a reinforcement area of 676 in^2 surrounding the fenestration. In our study, we sampled four 2- by 13-in. areas (104 in^2) located 1.5 in. from the edge of the fenestration for bacterial content after each procedure. Therefore, our sample size was 15.4% of the total area immediately contiguous to the surgical incision site (approximately 2/13 of the reinforcement area). The size of the area analyzed was limited by the method of bacterial isolation used and was as large as practical.

We found that in any fabric some bacteria become trapped in the interstices of the fabric. These bacteria cannot be removed by mechanical agitation. When a known number of bacteria are placed on a fabric, the percentage of bacterial entrapment varies, depending on the fabric. The nonantimicrobial control fabric used in this study normally retains 12 ± 4% of the input bacterial population when the bacterial isolation technique used in this study is used; *i.e.*, approximately 7/8 of the input bacterial challenge was recovered in control studies. Therefore, when the unsampled drape area and expected bacterial entrapment are taken into consideration, it is apparent that the number of bacterial isolates recovered in the study represents only a small portion of the potential pathogens that might be present in the area surrounding the surgical incision. The theoretical total number of bacteria that actually were present in the surgical field at the end of each procedure can be derived from the following formulas: (i) (CFU isolated per procedure/7) 8 = total theoretical bacterial count on the sampled area of the reinforcement corrected for bacterial entrapment; (ii) (CFU [corrected for bacterial entrapment] per procedure/2) 13 = total theoretical bacterial count present on the surgical field at the end of the procedure after corrections for bacterial entrapment and inclusion of the CFU on the unsampled area of the reinforcement.

TABLE 1. CFU recoveries from surgical drapes

Side of patient	Bacterial recovery (CFU) from:								% Bacterial reduction attributable to antimicrobial fabric[a]
	Antimicrobial swatches				Nonantimicrobial swatches				
	No.	Mean	Range	Median	No.	Mean	Range	Median	
Both	8,025	184	0–5,000	12.5	51,586	1,172	21–20,000	105	84.4
Left	3,382	78	0–2,500	3	26,240	596	0–10,000	52	87.1
Right	4,643	105	0–2,500	8	25,349	576	0–10,000	25	81.7

[a] Percent reduction = (CFU recovery from nonantimicrobial fabric − CFU recovery from antimicrobial fabric)/CFU recovery from nonantimicrobial fabric.

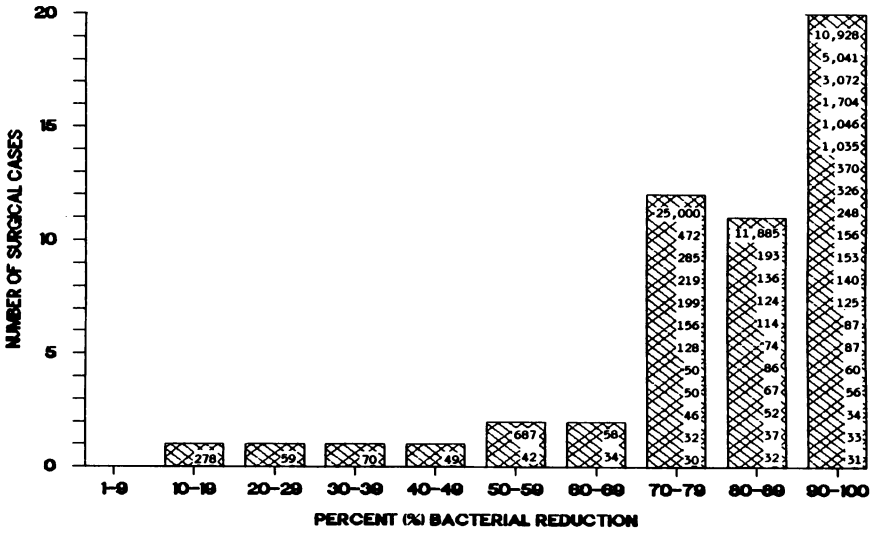

FIG. 4. Bacterial reduction percentage frequency. The individual numbers within each bar of the histogram refer to the actual number of total CFU isolated in the individual procedures analyzed. Each number refers to a single case demonstrating the indicated percentage of bacterial reduction.

These simple mathematical formulations supply a number that reflects the actual potential pathogen population present on the reinforced portion of the drape at the completion of a surgical procedure. The numbers of bacteria in the sterile field derived by using these procedures compared favorably with the bacterial counts found by Sampolinsky in his study on bacterial contamination in a sterile field (15).

Hooten et al. (8) reported that the length of a surgical procedure influences the postoperative infection rate. The differences in the duration of surgery as reflected in group 1 versus group 2 correlated well with their observations. The clinical data demonstrated that, as the time for a surgical procedure increased, the number of bacteria on the surgical field increased.

This double blind in vivo study demonstrated the effectiveness and established the efficacy of an antimicrobial fabric in which a broad-spectrum antimicrobial agent was bonded to the fibers. The antimicrobial fabric reduced the number of potential pathogens surrounding the incision by a substantial margin, independent of the bacterial challenge.

TABLE 2. Percentage of surgical procedures in which specific organisms were identified

Organism(s)	% of cases in which organism(s) was isolated
S. epidermidis	60
S. hominis	53.9
S. capitis	26
S. haemolyticus	26.9
S. warneri	11.1
S. cohnii	4.7
S. aureus	3.2
Staphylococcus sp.	7
M. luteus	39.6
Miscellaneous gram-positive bacilli	15.8
Pseudomonas sp.	6.2
E. coli	4.7
Miscellaneous gram-negative bacilli	3.1

The antimicrobial fabric was efficacious in clean, clean contaminated, and contaminated cases regardless of the bacterial challenge. No wound infections or adverse healing problems developed in any of the patients. Also, no allergic reactions were seen.

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