Evaluation of Effects on Elevated Levels of Normal Skin Bacterial Flora with Fabrics Treated with 3-(Trimethoxysilyl) Propyldimethyloctadecyl Ammonium Chloride

By W. Curtis White, Bioproducts Technology Manager, Dow Corning Corporation
Benny L. Triplett, Burlington Industries

Summary

Concern has been expressed that antimicrobial agents used on fabrics can affect normal skin bacterial flora and give rise to adapted or dominant species. This imbalance could have negative effects. This work was undertaken to: (A) evaluate the effects of fabrics treated with 3-(trimethoxysily) propyldimethyloctadecyl ammonium chloride (Shield Antimicrobial Treatment) on elevated populations of normal skin bacteria under an occlusive dressing and (B) evaluate the retrievable counts of the target bacteria associated with treated and nontreated fabrics.

Results show clearly that fabric treated with 3-(trimethoxysily) propyldimethyloctadecyl ammonium chloride exerted no untoward effects on the elevated skin target bacterial populations under the occlusive dressing when compared to the untreated fabric. Testing of the target bacterial flora in the fabric itself showed 99.96 to >99.99 percent reduction in the treated fabric as compared to the untreated fabric.

Key words: Antimicrobial, Skin, Textiles, Silane

Introduction

Treatment of fabrics with antimicrobial agents (AA) to inhibit bacteria and fungi associated with body/fabric odor has been commercially practiced for many years [2,9]. Concern that these agents could affect normal skin bacterial flora and give rise to adapted or dominant species and negative effects of these imbalances has been expressed.

The purpose of this work was to evaluate the effects of fabrics treated with 3-(trimethoxysily) propyldimethyloctadecyl ammonium chloride (SiQAC) Shield Antimicrobial Treatment) on elevated populations of normal skin bacteria under an occlusive dressing and the survival of bacteria in the treated fabric.[1]

Skin colonization by bacteria is a normal and healthy process. The number and types of organisms can vary greatly and shifts from healthy to unhealthy conditions can occur quite readily. Skin microflora are associated with odors and disease. The role of the skin as a transfer (person-toperson), object-to-object, or person-to-object) agent of microorganisms is also noted.

Shield Antimicrobial Treatment for this report is synonymous with BIOGUARD® Fabrics and with

Shield Antimicrobial Agent treated fabrics.

The skin is clearly the definer of its own flora. Variable factors such as secretory antibodies, lipidlipopreotein availability, desquamation, rupture or abrasion, humidity, pH, bacterial adherence, microbial potentiation or antagonism and external toxicants influence the nature of the bacterial flora.

Insights relating to the relations of AA to skin microflora can be gained from the voluminous literature on AA used in soaps. Marzulli and Bruch5 described the following risks associated with the contact of skin by AA soaps: 1) skin irritation and/or sensitization, 2) photosensitizations, 3) selective antimicrobial activity, 4) percutaneous absorption, 5) cross-sensitizations, 6) ability to be formulated and 7) photo allergenic responses. We would add the potential of AA to stimulate mutational or inductive adaptation to this list of risks. Depending on the durable nature of AA used on fabrics, some or all of the above risks could apply.

The durable nature, broad spectrum antimicrobial activity, and positive results of in-use odor suppression by sock textile fabrics treated with 3-(trimethoxysily) propyldimethyloctadecyl ammonium chloride has been reported by Gettings and Triplett.[2] Their work included a demonstration of activity against a variety of laboratory and skin isolate bacterial strains (Table I).

An AA activity on skin can be evaluated by quantitating its ability to prevent the increase in the population of normal skin flora which under controlled conditions occurs beneath an occlusive dressing. The simple maneuver of applying impermeable plastic film (SARAN® WRAP) reinforced with adhesive tape over areas of the forearm allows for the increased temperature and moisture that allows the normal skin flora to propagate from a level of hundreds of organisms/cm^2 to over a million/cm^2 in a 48 hour period. An effective AA will prevent such an increase.[4] This technique gives reproducible data and has the advantage of each individual serving as their own control when a proximal area is occluded without the test agent.

MATERIALS AND METHODS

Fabric Preparation

Shield Antimicrobial Agent (42% SiQAC in methanol) was used to treat 60/40 orlon/nylon sock fabric at 1% as is in a commercial sized paddle machine at a 30:1 liquor to goods ratio at 110°F allowing twenty minutes for dynamic exhaustion. Untreated control socks were exposed to the same conditions without the presence of the AA. After treatment, the socks were extracted in a centrifugal extractor and dried for twenty minutes at approximately 230°F in a forced-air tumble drier. The final step was a boarding process where the socks are mounted on a board (flat metal “foot and angle”) and subjected to 280°F steam for approximately eighteen seconds. This treatment is representative of industrial processes except that the 1% treatment level would generally be considered high. High, both from its surface reactive properties as well as from its ability to effect microbial reduction and the benefits of that reduction.

Effects on Skin Bacterial Flora

Ten healthy volunteers, none using soap containing antimicrobial substances, were studied as follows: Three areas of 25cm^2 each were delineated on each forearm. On one arm, one site was covered with only SARAN® WRAP and then adhesive tape and sites two and three were covered with a single 25cm^2 swatch each of untreated sock fabric and then covered with SARAN® WRAP and adhesive tape. On the other arm, sites were prepared as above except that the 25cm^2 swatches were treated with the AA SiQAC. After 48 hours of occlusion, quantitative bacteriological cultures were obtained using the following retrieval technique.

Retrieval Technique

The localized detergent scrub technique of Williamson and Kligman was used.[13] In this method, a 3.8cm^2 area of skin is outlined by a sterile glass cup into which one ml. of a fresh solution of 0.1% Triton® X-100 surfactant was added and stirred vigorously for one minute. This procedure was repeated with a second one ml. of Triton® and the two samples were pooled. Ten fold serial dilutions were made in 0.05% phosphate buffered Triton® and standard microbiological plate counts were made as below.

Media

Standard microbiological plate counts were made with Trypticase Soy Agar (T.S.A. – a general growth media), T.S.A. with Tween-80 and lecithin added as neutralizers and to enhance growth of lpophilic diptheroids and MacConkey's agar for tentative enumeration of gram (-) organisms. Plates were incubated at 35°C for 48 hours and then at room temperature for an additional 48 hours before counting using standard counting techniques.

Effect on Fabric Bacterial Flora

In a separate experiment, five healthy volunteers were selected as above. Sample mounting was done as above except that a treated fabric sample, an untreated fabric sample and a site with no fabric were used on each arm.

Retrieval Technique

The fabric samples were cut into four equal parts (6.25cm2) using sterile techniques. Each sample was shaken on a Burrell Wrist Action Shaker (10 setting) for three minutes in 100 ml. of phosphate buffer modified with 0.1% of Triton® X-100. Ten fold serial dilutions were made in a 0.05% phosphate buffered Triton® and standard microbiological plate counts were made as above. Baseline studies using test fabric seeded by padding with various levels of Klebsiella pneumoniae ATCC 4352 and Staphylococcus aureus ATTCC 6538 showed that retrievals of at least 99% of the seeded bacteria could be expected after three minutes of agitation as above.

RESULTS

Effects on Skin Bacterial Flora

The results are tabulated in Table II. Good occlusion was obtained and the SARAN® WRAP control sites all supported a good growth, 6.0 X 105 to 9.2 X 106/cm^2. No significant differences in species types were noted between the control site, the fabric control site or the treated fabric site. Therefore, for the purposes of this paper, we will deal only with total populations. Both the untreated sock fabrics and the sock fabrics treated with SiQAC allowed a growth of bacteria less than the control sites although colonial morphologies were essentially similar. On each individual, the bacterial counts on the control sock fabric sites and the treated sock fabric sites were roughly within the same logarithm as shown in Table III. Since bacterial flora of skin are considered log-normally distributed, statistical analysis must be done after log transformation. When this is done, no statistical difference can be seen between the sites tested with control sock fabrics and those tested with treated sock fabrics ($p=0.6$). Comparison of untreated sock fabric sites with control sites shows that the untreated sock fabric significantly suppressed the expansion of the target skin bacterial flora under SARAN® WRAP ($p=0.001$). Likewise, the treated sock fabrics significantly suppressed the target bacterial population ($p=0.001$).

Effects on Fabric Bacterial Flora

Data generated during this work are tabulated in Table IV. As in the previous experiment, good occlusion was obtained and the SARAN® WRAP control sites all supported a good growth, 5.8 X 105 to 4.6 X 106/cm^2. Variation between right and left arm samples was not significant. As previously, the bacterial species identification was not significantly variable so total counts are used in the calculations. Comparing the skin bacterial flora control with the retrievals from the untreated control fabric one can note the large number of organisms associated with the test fabric. Making a similar comparison with the SiQAC treated fabric, one notes that very few organisms were retrieved. Percent reductions comparing retrieval numbers from the control fabric and the treated fabric can be calculated by the formula:

$$\% \text{ Reductions} = \frac{\text{Control Fabric Count-Treated Fabric Count}}{\text{Control Fabric Count}} \times 100$$

Percent reductions ranged from 99.96 to > 99.99% for the SiQAC Treated fabric.

Discussion

The commercial utility of treating a fabric with an antimicrobial agent so that the negative effects of microorganisms such as odor, deterioration, defacement and the presence of medically significant microorganisms can be minimized has been practiced for many years. The ability to accomplish this without causing change of the normal skin bacterial flora should be of considerable value. Diffusing types of AA that can leave the fabric and contact the skin present an opportunity for microorganisms to adapt through mutational or inductive biochemical processes.[11] The practical effects of such a phenomenon have not been fully invested. Diffusing AA may also cause skin irritation and sensitization responses as well as have the potential for percutaneous absorption.

A unique technology, the AEGIS Antimicrobial Treatment based on 3-(trimethoxysilyl) propyldimethyloctadecyl ammonium chloride, that chemically reacts with fabrics and acts as an immobilized antimicrobial has been thoroughly described.[3,6,8,10] This treatment offers the unique advantages of minimizing the chance for toxicological interaction when in contact with the skin. Skin irritation and sensitization tests show that no untoward effects are observed from the neat chemical or from the fabrics treated with the chemical.1 Additionally, a thorough percutaneous absorption study of water solutions of the neat chemical (its most mobile chemical form) showed no untoward effects.[7] Data have also been presented that show the unlikelihood that adaptive processes are initiated in the presence of the AEGIS Treatment.[12]

The test fabrics in this study showed the ability to reduce elevated populations of natural skin flora (in vivo) when the organisms were associated with the fabrics yet they did not affect the organisms on the skin. These observations are consistent with the reported chemical and antimicrobial nature of SIQAC.[3,6,8] Data presented in the part of this work entitled, Effects on Fabric Bacterial Flora, show reductions of organisms consistent with self-sanitizing surface claims.

Both types of sock fabrics did alter the environment in the occluded system such that skin retrievals from the skin were suppressed when compared to the normal increase in population that occurs under SARAN® WRAP occluded skin. Data from the retrievals made from the socks support this variation and show that the fabric becomes the favored substrate for the bacterial population increase. This is probably accounted for based on the predicted rise in humidity of the fabric as compared to the skin.

Interpretation of the data allows us to conclude that the test sock fabrics treated with SiQAC (AEGIS Antimicrobial Treatment) do not exert an antibacterial action on skin flora greater than that of similar socks not treated. We can also conclude from the data that the treated fabrics act as a selfsanitizing surface showing at least 99.9% reduction of the target bacteria.

TABLE I

Effectiveness of Shield Antimicrobial Treatment Socks Against A Variety of Laboratory and Sock Isolate Organisms[1]

BACTERIA	% BACTERIAL REDUCTION[2]
Micrococcus sp. I	99
Staphylococcus epidermidis	96
Enterobacter agglomerans	90
Acinetobacter calcoaceticus	99
Micrococcus sp. II	100
Micrococcus sp. III	99
Staphylococcus aureus (pigmented)	99
Staphylococcus aureus (nonpigmented)	99

[1] Adapted from Gettings, R.L. and B.L. Triplett$_2$

[2] Percent reduction as measured against an untreated control sock using AATCC-100 Test Protocol (Padding Test)

TABLE II

TOTAL COUNTS BACTERIAL DENSITY/CM^2

48 Hours Occlusive

Left Arm				Right Arm		
Subject	SARAN® Control	CS	CS	ST	ST	ST
1	$6.0x10^5$	$4.0x10^4$	$4.4x10^4$	$1.8x10^5$	$2.8x10^4$	$4.4x10^4$
2	$3.0x10^6$	$1.0x10^6$	$1.6x10^6$	$2.0x10^6$	$1.4x10^6$	$2.1x10^6$
3	$9.2x10^6$	$4.7x10^4$	$6.2x10^4$	$1.0x10^5$	$1.5x10^6$	$1.2x10^6$
4	$8.0x10^5$	$1.4x10^3$	$5.2x10^3$	$2.4x10^3$	$9.2x10^3$	$2.3x10^4$
5	$9.0x10^5$	$2.8x10^5$	$3.2x10^5$	$7.2x10^4$	$1.0x10^5$	$2.4x10^4$
6	$1.2x10^6$	$2.6x10^5$	$3.2x10^5$	$4.4x10^5$	$1.0x10^5$	$2.4x10^5$
7	$8.0x10^5$	$1.0x10^5$	$2.2x10^5$	$1.0x10^4$	$7.0x10^5$	$2.0x10^5$
8	$6.0x10^5$	$4.0x10^5$	$1.0x10^4$	$4.2x10^3$	$1.1x10^4$	$3.0x10^4$
9	$2.2x10^6$	$5.6x10^4$	$7.2x10^4$	$1.1x10^5$	$7.0x10^5$	$2.0x10^5$
10	$1.1x10^6$	$3.2x10^4$	$1.0x10^4$	$1.0x10^4$	$2.2x10^4$	$1.0x10^5$

CS = Control Sock; no antibacterial substance

AS = Shield Treatment

TABLE III

LOG TRANSFORMATIONS
BACTERIAL DENSITY LOG/cm^2
48 Hours Occlusive

Subject SARAN® Control Control Sock SYLGARD® Treatment

1	5.3981	4.2512 4.2754	5.1514 4.1905 4.2754
2	6.1995	6.0000 6.1445	6.1585 6.1380 6.1622
3	6.8318	4.2951 4.4169	5.0000 6.1413 6.1318
4	5.6310	3.1390 3.3311	3.1738 3.8414 4.1698
5	5.7943	5.1905 5.2089	4.5248 5.1271 4.1738
6	6.1318	5.1820 5.2089	5.2754 5.0000 5.1738
7	5.6310	6.0000 5.1679	4.1271 5.2089 3.0000
8	5.3981	4.2512 4.0000	3.2680 4.1288 4.1995
9	6.1660	4.3631 4.2089	5.1288 5.5012 5.1585
10	6.0000	4.5258 4.0000	4.1585 4.1660 5.0000

TABLE IV

TOTAL COUNT BACTERIAOLOGICAL LEVELS AS RETRIEVED
FROM 48-HOUR OCCLUDED SKIN MOUNTED FABRICS

Test Subject	SARAN® Control[1] (skin retrieval) organisms/cm^2	Untreated[2] Control Fabric (fabric retrieval)[3] organisms/cm^2	SiQAC Treated[2] Fabric (fabric retrieval)[3] organsisms/cm^2
1) Right Arm 1) Left Arm	$2.1x10^6$ $1.8x10^6$	$1.6x10^6$ $1.2x10^6$	380 100[4]
2) Right Arm 2) Left Arm	$6.8x10^5$ $8.1x10^5$	$6.1x10^5$ $7.0x10^5$	100 100
3) Right Arm 3) Left Arm	$4.6x10^6$ $3.8x10^6$	$3.7x10^5$ $2.4x10^6$	680 720
4) Right Arm 4) Left Arm	$8.4x10^5$ $5.8x10^5$	$7.3x10^5$ $4.1x10^5$	100 100
5) Right Arm 5) Left Arm	$1.5x10^6$ $1.8x10^6$	$9.2x10^5$ $1.2x10^6$	210 480

1 Method of Williamson and Kligman13
2 Shaker Retrieved
3 Average of three pour plates
4 Sensitivity of the retrieval technique

SYLGARD® Antimicrobial Treatment is a registered trademark of Dow Corning Corporation.
BIOGUARD® textiles is a registered trademark of Burlington Industries.
SARAN® Wrap brand plastics film is a registered trademark of the Dow Chemical Company.
TRITON® Surfactant is a registered trademark of Rohm and Haas Corporation.

ACKNOWLEDGEMENTS

The authors thank Mr. Pat Walters and Mr. Richard Gettings of Dow Corning Corporation for their help in the design of the Effects on Skin Bacterial Flora part of this work. We also thank Albert M. Kligman, M.D., Ph.D. for the design and implementation of this same part of the work.

REFERENCES

1. “A Guide to Antimicrobial Agents, Dow Corning® 5700 Antimicrobial Agent”, 1985. Dow Corning Corporation, Midland, Michigan, Form No. 19-022C-85.

2. Gettings, R.L. and B.L. Triplett, 1978. “A New Durable Antimicrobial Finish for Textiles,” In: Book of Papers, AATCC National Conference. 46-51

3. Malek, J.R. and J.L. Speier, 1982. “Development of an Organosilicone Antimicrobial Agent for the Treatment of Surfaces,” In: J. of Coated Fabrics. 12:38-46.

4. Marples, R.R. and A.M. Kligman, 1974. “Methods of Evaluating Topical Antibacterial Agents on Human Skin,” In: Antimicrobial Agents and Chemotherapy, Am. Soc. For Micro. 5:3:323-329.

5. Marzulli, F.N. and M. Bruch, 1981. “Antimicrobial Soaps: Benefits Versus Risks,” In: Skin Microbiology, Relevance to Clinical Infection”, Springer-Verlag, New York. 125-148.

6. McGee, J.B., J.R. Malek, and W.C. White, 1983. “New Antimicrobial Treatment for Carpet Applications,” In: Am. Dyestuff Reporter. 16-18.

7. Sidiqui, W.H., J.R. Malek, et al, 1983. “Percutaneous Absorption of an Antimicrobial Organosilicon Quaternary Ammonium Chloride,” In: J. of Appl. Tox. 3:3:146-149.

8. Speier, J.L. and J.R. Malek, 1982. “Destruction of Microorganisms by Contact with Solid Surfaces,” In: J. of Colliod and Interface Sci. 89:1:68-76.

9. Vigo, T.L., 1976. “Antibacterial Finishing of Textiles,” In: Chemtec. 455-458.

10. Walters, P.A., E.A. Abott, and A.J. Isquith, 1973. “Algicidal Activity of a Surface-Bonded Organosilicon Quaternary Ammonium Chloride,” In: Applied Microbiology. 25:2:253-256.

11. White, W.C. and S.F. Hayes, Sept. 1982. “A Feature With a Future – SYLGARD® Treatment for Carpeting, “ In: Fall Technical Symposium, Carpet and Rug Institute, Dalton, GA.

12. White, W.C. and G.M. Olderman, May 23-24, 1984. “Antimicrobial Techniques for Medical Nonwovens: A Case Study,” In: Book of Papers, INDA, Association of The Nonwoven Fabrics Industry, New York, N.Y., 12th Annual Technical Symposium. 13-46.

13. Williamson, P. and A.M. Kligman, 1965. “A New Method for the Quantitative Investigation of Cutaneous Bacteria,” In: J. Invest. Derm. 45:6:498-503.