Antimicrobial Techniques for Medical Nonwovens: A Case Study

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Note: This paper documents extensive research and developmental work done jointly by Dow Corning Corporation and the American Convertors Division of American Hospital Supply (now part of Baxter healthcare). Curtis White is now CEO and Director of R&D at ÆGIS Environments and Dr. Olderman has retired.

Introduction

The nonwovens industry is challenged by the presence of microorganisms and the negative effects they cause. Deterioration, defacement and odors are all dramatic effects which occur from the microbial contamination of nonwovens. Nonwovens can also act as a "harbor" as most they offer ideal environments for medically significant microorganisms. The ability to make nonwovens resistant to microbial contamination has advantages in many applications and market segments. This is especially true in medical markets where nonwovens have already contributed a degree of aseptic sophistication beyond historically used linens.

Nonwovens used in medical applications have unique microbial problems and their control is a complex microbiological task. Use of nonwovens in the United States medical community has greatly expanded in recent years as evidenced by the fact that over half of the drapes used in surgery are nonwovens. The microbiological integrity of nonwovens has been the object of numerous studies ranging from the sterilization of nonwovens to the evaluation of the barrier properties of nonwovens. Test data generated with nonwovens generally support the fact that nonwovens contribute positively to the reduction of microorganisms in the medical environment. This contribution has been part of the medical communities awareness of the benefits of and actions aimed at improving the hygienic nature of their environment as they take steps towards asepsis.

History

The surgical arena provides a valuable model for illustrating the medical communities' challenges as regards asepsis. The first surgery may have occurred nearly twelve thousand years ago. Laws regarding the performance and liability of surgeons were included in the code of Hammurabi in 1700 B.C. with mention of such retribution as the surgical removal of the hand of the physician whose patient lost an eye or succumbed to the procedure.

The first use of the word inflammation appears to date back about twenty-five hundred years and is mentioned in three tablets from Assurbanipal's library.[1] The ancient Greeks mistook infection as a "...good and natural course of events" and poured wine into wounds to help them heal.[2] It is only coincidental that the disinfecting properties of wine are based on a chemistry very similar to that of Lister's phenol, but we come full circle when we recall that Pasteur's work on preventing wine spoilage led to Lister's theories.[3] It was not until the last quarter of the nineteenth century, after Semmelweiss had died, Oliver Wendell Holmes had written of the risks of bacterial contamination, and Lister had laid the ground work for surgical asepsis, that the first surgical drapes and apparel came into use. Three-quarters of a century after Neuber and Robb[4] initiated the use of linens, the first nonwoven drapes were introduced in the United States, and a second tier of wound isolations (asepsis) was attained.

Because the medical literature is replete with studies on the epidemiology, rate and cost of post-operative infection, this review is intended to bring into focus the position stated on aseptic barrier materials and their impact on infection. While there may be little agreement on the specifics of such factors as infection rate and costs, and the relative importance of the numerous individual parameters and complex interactions which impact wound infection, it is putative that

the strict observation of sterile technique and the proper application of drugs and devices can reduce infection rate. One excellent review[5] refers to the five D's or O.R. infection control: discipline, defense mechanisms, drugs, design and devices, and outlines a cogent basis for reducing risk of infection.

Summary of the Literature

In 1952, Beck proved that bacteria pass through layers of absorbent linen with "instantaneous rapidity", but a nonwoven material, treated with a water repellent finish, resisted bacterial transmission and appeared to be "ideal as a bacterial barrier[6].

Twenty-six years after this remarkable discovery, Dr. Beck was still exhorting the reader to employ a draping system that would resist the passage of aqueous solutions[7]. During that quarter of a century, as the quality of nonwoven materials were improved and the variety of surgical drapes and apparels for use in all types of operations was expanded, several other investigators began to evaluate and compare these new single use nonwovens to conventional absorbent 140 thread count linen (muslin) as well as the new more recent 270 plus thread repellent woven product (Pima).

In 1964, Sweeney reported that the nonwoven drape he tested on nearly twelve thousand infant deliveries "might serve as a more effective barrier to pathogen migration than the traditional cotton drape", and adjudged the disposable nonwoven drape to be a superior aseptic barrier to the bacterial migration in obstetrics patients.[8]

In 1969, Peter Dineen showed the superiority of disposable nonwovens to linens in the reduction of air borne contamination by 90%, and in 1973 he demonstrated the prevention of bacterial penetration in liquid media "purely on the basis of the water repellency" of the nonwoven material. [9, 10]

Again in 1973, the superiority of nonwoven materials to muslin was demonstrated by Alford, et,al., at Indiana University. They found a 33% reduction in colony counts on the surface of gowns after 30 minutes of exercise.[11]

The need for this quality in a gown was supported by the work of Charnley and Eftekhar who in 1969 reported that "organisms shed by the surgeon's body may penetrate operating gowns, and by direct contact, infect operative wounds."[12]

Several other studies probing this premise followed in rapid succession. In 1979, Ha'Eri and Wiley used human albumin microspheres as tracer particles to demonstrate that nonwovens were superior to muslin in preventing bacterial penetration and reducing the risk of wound contamination. In one-hundred-ten orthopedic operations, not one tracer particle which had been sprayed on the patient's skin and the surgeon's chest and shoulders, was detected in the wound when nonwovens were used. The number of tracer particles observed when muslin was employed varied with the length of the procedure and the degree of physical strength during surgery, but all wounds were contaminated.[13]

Whyte, et.al., found in laboratory studies that the use of nonwovens reduced surface count contamination by fifty to sixty percent over closely woven "ventile cloth," which is the British version of the pima fabric.[14] Further support came from the work of Hamilton, et.al., published in 1979. Under "clean room" O.R. conditions with five-hundred-ninety-five orthopedic cases, they showed that viable organisms from the surgical team, which they claimed can account for twenty percent of wound contamination, penetrated one out of every ten gowns comprised of closely woven repellent Pima and conventional muslin fabrics. In four out of nineteen wounds (21.1%) that were contaminated, the identical organisms were found on the external surface of the gowns as well.[15]

Finally, J. Moylan, *et al.*, in 1975 and again in 1980, reported similar results on linen and nonwoven gowns. In the earlier study, Moylan reported external gown contamination increasing from 23% to 76.5% on the nonwoven gown compared to 85.2% increasing to 94.4% with muslin over the period of one to four hours of surgery in one hundred cases.[16] In 1980, Moylan and coworkers published clear clinical evidence of the superior efficacy of a nonwoven gown over both muslin and Pima repellent treated linen gowns. After an eighteen month study with 2,253 consecutive surgical operations in two different hospitals, the infection rates reported were: 4.75%

for Pima gowns versus 1.83% for the nonwoven in Hospital A, and 8.2% for muslin versus 3.07% for the nonwoven in Hospital B.[17]

Hartman also reported the reduction in post-operative infection rates from 6.5% to 1.0% at Gillette Hospital when new aseptic techniques were introduced in combination with the application of nonwoven drapes over a seven year period.[18]

These results are not surprising in the light of recent laboratory testing reported in the literature. For example, H. Laufaman, et.al., compared nonwovens to Pima fabrics under a static pressure head of liquid containing bacteria. They reported that polyethylene reinforced nonwovens may be considered suitable for lengthy, wet operations and found no significant difference in performance between a reinforced nonwoven system and the treated Pima cotton.[19]

In April 1980, Schwartz and Saunders reported on both lab testing and clinical comparisons of muslin and Pima cotton as well as two different nonwovens.

They found that the treated Pima and the two nonwovens were effective barriers and suggested that, in their opinion, there would be a reduction in infection with the use of any of these three materials.[20]

While a great number of factors influence the rate of post-operative infection, certainly the reduction in wound contamination is one of the most critical. Davidson, et.al., studied fifteen different variables of techniques with 1,000 patients and reported that "a wound which gave a positive culture at the end of the operation has a 47.9% greater chance of becoming infected than a wound found to be sterile at closure."[21] One philosophy that these investigators, and others who have evaluated surgical materials, agree upon is that muslin is unacceptable as a bacterial barrier material. Indeed, it has been stated that muslin will not bar passage of bacteria either wet or dry, even for a few minutes. Thus the risk of wound contamination is significant when muslin is used. In fact, the Technical Standards Committee of the AORN has published a brief, but comprehensive set of standards for surgical

drapes and gowns, which require blood and aqueous fluid resistance.[22]

There is less agreement on which of the other surgical drapes and gown materials on the market today is more or less suitable. You have seen a review of most of what has been done to evaluate the performance in terms of wound infection under controlled clinical conditions, but there is still no consensus on which of many laboratory, physical or microbiological tests does the best job of evaluating them. In fact, there is no clear definition on the specific variables such as time, liquid typed, pressure, stress and so forth that should be tested and at what level. The only direction on that topic was developed by an ad hoc committee of industry in conjunction with the American College of Surgeons. This work has been continued by INDA, the Association of the Nonwovens Industry, and AAMI in response to the challenge that surgical materials should be "impervious to the penetration of bacteria under the usual conditions of use."

Newer materials are being developed to respond to the needs of the surgical team. These materials offer more comfort and better performance with little or no sacrifice to their efficacy in restricting the passage of bacteria. In fact, recently a third tier of aseptic barrier materials, one which contains an antimicrobial agent, has been introduced for use in surgery. This material is directed at reducing the amount of contamination transferred to the wound from the surgical team, through scrub clothing and gowns, onto the sterile field or by the endogenous bacteria, deposited on the surface of the drape during surgery and then transferred into the subcutaneous region of the wound where it can increase the risk of infection.[23, 24]

This third tier of aseptic barrier materials addresses itself to the critical dose variable in the Altemeier and Culbertson equation which expresses that, "wound infection is the unfavorable result of Dose of Bacteria times Virulence divided by the Resistance of the Patient."[25] Analysis of this formula shows that the dose variable is the one variable, i.e. when bacteria were present at closure the risk of nosocomial infection increased significantly.[26] Robeson's work with skin graft patients clearly showed the

importance of dose as expressed by infectious threshold levels of greater than 105 and 106 bacteria per gram of tissue. The logic and evidence that reducing the dose (level) of microorganisms in the field of the wound site will reduce the risk of post-operative infections is irrefutable.

The desirable performance characteristics of this third tier antimicrobial nonwoven drape are that the antimicrobial nonwoven drape reduces the level of bacterial contamination, controls and/or kills the bacteria commonly associated with surgical wound infections, takes an active role in maintaining an aseptic field at the wound site, that the antimicrobial is safe to the staff and the patient, that the fabric's antimicrobial activity is unaffected by common sterilization procedures, and that the fabric retains all of the positive handling and appearance characteristics desired by the OR and surgical staff. In 1978, American Convertors and Dow Corning Corporation undertook the challenge of developing a fabric that met the above needs.

This paper discusses the microbiological techniques employed in the development of the American Convertors ISO•BAC Antimicrobial Fabric (AC-AM Fabric) which utilized Dow Corning 5700 antimicrobial agent (now known as AEM 5700 Antimicrobial) (silanequat), the properties of this unique antimicrobial agent, the safety profile of this chemistry and this state-of-the-art fabric, as well as the effectiveness of the AC-AM Fabric.

The Chemical Technology

The antimicrobial activity of certain silane-modified surfaces was discovered during a screening project in which the minimum inhibitory concentrations (MIC) for bacteria were being determined for various Dow Corning products and research materials. Repeat testing in the same glassware revealed that the glassware itself had become antimicrobial. Continued investigation led to a series of U.S. patents and publications covering this class of materials as broad-spectrum algicides, bactericides, a fungicides when applied to solid substrates. Further examination of this phenomenon and the chemistry involved resulted in the preparation of a single material which was more extensively evaluated. This material is chemically, 3-trimethoxysilyl-propyloctadecyldimethyl ammonium chloride (silanequat).

AEM 5700 offers users the following features:

- Good durability - In the presence of moisture, AEM 5700 antimicrobial agent imparts a durable, broad spectrum, biostatic surface finish to a wide range of substrates. It is leach resistant, non-migrating, and is not consumed by microorganisms. Broad spectrum activity - Effective against gram positive and negative bacteria, fungi, algae, and yeasts.

- Increased efficiency - Through proper application, durable bacteriostatic and fungistatic and algistatic surfaces can be attained with a minimum amount of Dow Corning 5700 antimicrobial agent.

- AEM 5700 antimicrobial agent can be applied to organic or inorganic surfaces as a dilute aqueous solution to give 0.1-1.0 percent by weight of active ingredients. Aqueous solutions can be prepared by simply adding the antimicrobial agent to water while stirring.

- Surfaces can be treated with the aqueous by dipping, padding, or by automated spraying until adequately wet, or applying by foam finishing techniques.

After applying the antimicrobial agent, the surface should then be dried to effect complete condensation of silanol groups at the surface and to remove water and/or traces of methanol from hydrolysis. Optimum application and drying conditions such as time and temperature should be determined for each application before use in a commercial process.

The first commercial application, on men's socks, helped prevent microbially caused deterioration and defacement and reduced sock odor associated with the proliferation of microorganisms. A paper by Gettings and Triplett presented conclusive evidence that the antimicrobial feature provided a significant reduction in sock odor and that the protection afforded by the treatment was not significantly diminished even after repeated launderings.[27] Mechanisms of attachment to surfaces, general treatment phenomena, and performance profiles

have also been previously presented by Malek and Speier and will not be detailed in this paper.

AEM 5700 is registered with the EPA (#64881-1) for use as a pesticide on numerous substrates. This chemistry has also been reviewed by the F.D.A. and is listed as a modifier of medical devices under the 510(k) procedures.

Safety Profile

Safety considerations regarding the use of an antimicrobial on a surgical drape fenestration offers a model where the severity of risk to health is magnified beyond the risks encountered on less critical goods such as CSR wraps, table covers, or the like. This is especially relevant as one remembers that antimicrobials, by definition and function, inhibit and/or kill living things. The mode of biological involvement needs to be fully understood so that a proper balance between risks and benefits can be made.

The ability of the silanequat to chemically bond to the nonwoven substrate and still provide for the broad spectrum control of microorganisms made it well suited to the safety challenges encountered in this application, but a large body of toxicological data still needed to be generated. Considering the life history of the fabric, the key toxicological tests revolved around the toxicological profile of the silanequat itself and the AC-AM Fabric in use near an open wound site.

The following studies have been conducted with the silanequat:

(a) acute oral
(b) acute ocular
(c) acute and subacute dermal
(d) acute vapor inhalation
(e) primary skin sensitization and irritation
(f) sub-acute vaginal irritation
(g) four-day static fish toxicity
(h) teratogenic evaluation
(i) sub-acute human wear test (socks)
(j) human repeated insult patch test,
(k) in-vitro Ames Microbial Assay with and without metabolic activation
(l) in-vitro mammalian cell transformation in the presence and absence of exogenous metabolic activation,
(m) in-vitro Host-Mediated Assay
(n) a percutaneous absorption study.

Although certain handling cautions are indicated by data from the above tests, no untoward effects are notable regarding treated substrates.

The AC-AM FAbric was further subjected to the following pre-clinical biocompatibility tests which are considered appropriate for skin contact medical products:

(a) Tissue culture (cytotoxicity), to determine if a tissue culture medium (with serum) eluate of the test material can induce a cytopathic effect on monolayers of human (WI-38) cell
(b) Acute systemic toxicity to evaluate the potential of a single injection of an extract of the test material to produce a systemic toxicity response
(c) Intracutaneous irritation to evaluate the potential of a single injection of the test material extract to induce tissue irritation
(d) Eye irritation to determine the response of the rabbit eye to the instillation of specific extracts of the test material
(e) Hemolysis to determine if a substance can be extracted from the material which is capable of inducing hemolysis of human red blood cells
(f) Human Repeated Patch Test to determine if the test material is capable of inducing skin irritation and sensitization under controlled patch test conditions
(g) Extensive leachability studies to evaluate the durability and non-leaching potential of the chemically modified fabric when exposed to copious amounts of physiological saline, water and simulated human sweat.

The final results of these biocompatibility studies indicate that AC-AM Fabric is non-toxic, non-irritating and non-sensitizing to human skin, and has a permanent antimicrobial capacity which cannot be extracted in use. These pre-clinical studies provide sufficient information to allow us to predict the biocompatibility of the finished products and support their safe clinical use. As such, AC-AM Fabric is considered safe for use in surgery. Four years of clinical use with no untoward effects also supports the suitability of the AC-AM Fabric for its intended use.

Routine quality assurance specifications were also put into place to assure uniformity,

durability, and efficacious nature of the AC-AM Fabrics.

Efficacy Profile

Parallel to the safety work, a considerable body of microbiological efficacy data were being generated. To support the effectiveness of this third tier "active nonwoven" a variety of microbiological tools were utilized. These include: in-vitro tests, Scanning Electron Microscopy (SEM) work, and clinical evaluations. The purpose of these tests are to support claims relating to the reduction of microbial dose on the drape in the vicinity of the wound. The AC-AM Fabric kills the bacteria commonly associated with surgical wound infections and takes an active role in maintaining an aseptic field at the wound site. The antimicrobial surface serves to isolate the wound from bacterial transfer from the drape surface. The antimicrobial component of the AC-AM Fabric is chemically bonded, safe for use in surgery, and does not lose its effectiveness when sterilized, stored, or handled during the manufacturing procedure or in surgery.

Test Techniques - In vitro Barrier Fabric

Initial efforts in the development of the antimicrobial nonwoven fabric were aimed at using 3-trimethoxysilyl-propyldimethyloctadecyl ammonium chloride on a barrier drape to provide a more hygienic field. Classical microbiological methods did not work to demonstrate efficacy because solution activity as demonstrated in the Minimum Inhibitory Concentration Test (MIC) was irrelevant to a bound antimicrobial and since the anti-microbial agent did not leach the zone of inhibition test was not appropriate[30] and padding tests[31] did not have utility without the use of very sophisticated wetting agents. Linking these laboratory tests to "real world" performance was nearly impossible.

MIC Tests (Table I)

Although the silanequat is not an efficient solution active antimicrobial, the obligatory MIC tests have been run. Results of these tests show clearly the broad spectrum activity of the silanequat. Interpolation of these data to the real world is dangerous since the chemical nature of the silanequat makes any water solution testing dynamic. Chemically, the silanequat in water is constantly bonding and unbonding with itself and any reactive surfaces available. This "living polymer" nature of the material in water solution makes MIC data extremely variable depending on the design of the test protocol and the handling of the test solutions.

Zone of Inhibition Test

The zone of inhibition test, when a zone is produced, shows that the antimicrobial is not durable. This increases the risk of toxicological involvement and the risk of mutational or inductive adaptation phenomena being manifested. Although the silanequat does not give a zone of inhibition, encroachment of the test organisms onto the test surface is eliminated. The fungal control demonstrated in Figure 1 below clearly shows this benefit as compared to a traditional leaching type of antimicrobial.

FIGURE 1 Results: AATCC Method 30; AEM 5700 treatment, conventional antimicrobial treatment, and untreated control. Washed[1] and unwashed.

1. Five home launderings using AATCC detergent and protocol.

Note the durability evidenced by the continued activity of the silanequat after five home launderings of the cotton fabric whereas the traditional leaching type of antimicrobial treated surface no longer shows any protection against the test organism. This fungal activity and durability are well suited for many nonwoven applications. Table II shows typical results from the AATCC-30 Fungicide Test Protocol and further supports this important property.

Padding Tests

The utility of padding type protocols to testing the original silanequat treated barrier fabric seemed appropriate except for the hydrophobic

nature of the treated fabric. This introduced considerable error into the testing and modification of the AATCC-100 antimicrobial test protocol to include sophisticated wetting agents was necessary. Padding tests are useful as an indicator of surface antimicrobial activity but are difficult to run reproducibly and are extremely operator sensitive. Typical results using the AATCC-100 protocol plus re-wetter are shown in Table III. A number of variations of this test have utility in understanding the antimicrobial activity of nonwovens and will be discussed later.

Dynamic Shake Flask Test

To overcome the testing problems associated with the hydrophobic nature of the test surface and yet maintain some linkage to "real world" dynamics, American Convertors, using a modification of the classical rotating tube test, developed a dynamic shake flask test. This test has been modified as follows by Dow Corning: The test utilizes a 150ml. Ehrlenmeyer flask in which 5 ml. of a liter of 1×10^5 to 3×10^5 CFU/ml. (as Colony Forming Units) of test organism is added to 70 ml. of phosphate buffer or other test solutions and a measured amount of test fabric. This system is then placed on a Burrell Wrist Action Shaker for a representative time period. Zero time and test time control and treated samples are then compared for percent reduction. Results from this testing showed that the fabric could be treated durably and uniformly with Dow Corning 5700 and that the fabric was effective against both gram negative (*Klebsiella pneumoniae*) and gram positive (*Staphylococcus aureus*) bacteria. Data generated using this test protocol can be seen in Table IV. Clinical isolates were used as the test organisms. Note the effective range was from 93.6% - 99.9% reduction for these organisms commonly found in hospital situations. Since the innoculum control showed the organisms to be healthy, one could assume that those organisms that showed reduction with the untreated controls were sensitive to some component of the fabric or were trapped within the fabric and therefore, not recovered.

Barrier Fabric Discussion

Although these results were encouraging, a marketing reality had to be faced in that the marketplace preferred a drape that had an absorptive fenestration. Absorptive fenestrations had been avoided by American Convertors because of the potential reservoir or organisms that could build up during typical surgical procedures. Armed with a safe antimicrobial system, consideration of an absorptive fenestration could be made with the risk of increasing the microbial dose minimized or eliminated. Fabric design was optimized using technology jointly developed with Burlington Industries but safety and microbiological testing still presented a challenge.

Test Techniques -
In Vitro Absorptive Fabric

Fabric design, application procedures, safety, and antimicrobial efficacy are critical to the utility of the final nonwoven product. Once the fabric design, application procedures, and safety considerations had been completed, efficacy evaluations of the AC-AM Fabric were undertaken.

Padding Tests

As described earlier, various modifications of the AATCC-100 test have been used to demonstrate the effectiveness of the AC-AM Fabric. Listed in Table V are results from a fluid compatibility test run using buffered phosphate, saline, and serum. The *K.pneumoniae* microbial dose was added to each of the test fluids and then aliquots were applied to treated and control fabrics. Results were very uniform and confirm that microbial loads from such fluids are readily controlled on the AC-AM Fabric.[33]

The above work was extended in an attempt to compare the antimicrobial effectiveness of several types of fabrics where reinoculated blood and defibrinated blood were used as the carrier mediums. The test organism was *Klebsiella pneumoniae* ATCC 4352. Innoculum level was 1.5×10^5 CFU/ml. Note that results in Table VI (whole blood testing) show a rather uniform loss of retrievability of the test organisms irrespective of test substrate. This was attributed to the effects of the blood clotting through time removing the organisms from retrieval and in fact killing most of them. The killing effects of the blood and also defibrinated blood were further studied by following the course of an insult of *K. pneumoniae*,

on linen. (Table VII) Results show clearly the die-off effect in the whole blood, whereas no significant effect can be seen with the defibrinated blood through the six hour test period. Note that the linen inoculated with the contaminated blood extended the life of the test organisms. The significance of the 100% reduction in 5 min. on the D Sample (ISO•BAC, Table VI) needed to be established so an additional test was run using defibrinated blood. Table VIII contains their results of this testing. The clear value of reducing microbial dose level is illustrated in these results. Whereas neither the linen (A) nor the two untreated nonwovens (B and C) showed any reduction of the test organisms through two hours the ISO•BAC Fabric showed a 59% reduction in 30 minutes and 72% reduction after two hours. These tests were very rigorous in terms of organic load and microbial load and yet bacterial dose levels were significantly reduced.

To expand on our understanding of the influence of fluids a padding test was undertaken using Clark-Lubs solution (KH_2PO_4/NaOH) and the Acta "Sweat" as pre-wetting agents and the carrier fluids for *Staphylococcus epidermidis*. (Table IX) Again, the results support the excellent antimicrobial activity of the AC-AM Fabric.34

One of the most thorough studies utilizing the AC-AM Fabric was conducted by W.U. Faber et.al. at the West German Institute for Hospital Hygiene and Infection Control.35 Their test protocol, a swatch pad test, utilized linen, Molnlycke, and ISO•BAC Fabrics, four bacterial strains (*S. aureus, Streptococcus faecalis, K. pneumoniae, and P. aeruginosa*), three solutions used to stimulate O.R. conditions (buffered water, physiological saline, and blood serum), and five retrieval time intervals (0, 15 min., 30 min., 60 min., and 120 min.). The innoculum concentration was 1 x 105 to 1 x 106 CFU/ml. inoculated onto a 5 x 5 in. test fabric swatch. "All test bacteria and solutions indicate that the highest bacteria reduction occurred with the ISO•BAC Fabric in all cases. It is obvious that in linen and non-textile drape material, the bacterial kinetics show only minor differences, whereas, in ISO•BAC, the bacterial count is significantly lower when compared to the initial count. It is assumed that when using ISO•BAC materials, a transmission of bacteria by means of the draping material is prevented to the highest possible extent."

Pulse Height Analysis [36]

The effectiveness of AC-AM Fabric in reducing and controlling pathogenic organisms (commonly occurring in the operating theater) is of prime importance. Therefore, tests to evaluate the performance of AC-AM Fabric against *Escherichia coli* and *Staphylococcus aureus* were performed using Sontara and a suspension of the test organisms alone as a control. Two suspending media, saline and phosphate, were used and each combination was treated in triplicate.

In previous experiments, agar plate counts to establish the reduction of viable bacteria had been the method of choice. In this study, another approach using a modified particle counter was employed. This procedure takes samples from the flasks containing the swatches and bacterial control and processes the samples through the particle counter instead of making plate counts. The particle counter is modified to focus on bacterial sized particles, counts and sizes particles aspirated through an orifice, automatically recording the data on the numbers and size of the particles in 50 ml aliquots of the sample.

These data are presented both as a print-out of the total counts and an oscilloscope tracing showing the numbers of particles in various channels which represent the sizes of the particles. The sum of particles seen in a peak channel (each sized particle) can be compared with any other channel. For example, if there were 100 particles of a certain size in one channel and 10 particles of another size in the second channel, the height or peak of the first channel would be greater than the second channel, yielding valuable differentiation with respect to the size of the particles in the sample. In this study particles are equated with bacterial particles after an appropriate correction is made for background particles.

These data - total particle counts and particle size - can be used to interpret the effectiveness of a germicide against a bacterial population. An effective germicide must reduce the numbers of bacteria in contact with it by inflicting damage on the bacteria. The particle counter provides this information. The print out records the total counts

of bacterial particles from the test samples and provides a basis for determining whether a reduction in total bacteria occurs. The oscilloscope tracing shows two facts. First, it shows the distribution of different sized particles. Usually a bacterial cell which has been affected or damaged by a germicide has a different size than the control culture and this is seen on the oscilloscope tracing. This tracing also reflects the total number of particles in the 50 ml sample by the area under the combined peaks as well as the number of particles of each size. The print-out and the oscilloscope tracing thus yield information on the reduction of bacterial population and the damage done to the bacterial cells - i.e., the effectiveness of the germicide.

This particle counting and sizing method gives more information than agar plate counts because it gives an indication of bacterial damage as well as reduction of bacterial populations. In addition, the instrument offers other advantages. The instrumented method yields immediate results - one minute after taking each sample, provides a permanent record, and is completely objective.

In this study limited parallel plate counts for viable bacteria demonstrated the close parallel in results from the two procedures. The particle counts always exceeded viable bacterial counts but % reduction of bacterial populations was very similar using both procedures. This finding supports the validity of the particle counter in this type of testing.

The inoculum of *Escherichia coli* and *Staphylococcus aureus* was adjusted to 1 x 106 per ml. The suspending media were physiological saline (Abbotts injectable) and phosphate solution (35g. monobasic potassium phosphate/liter at pH 7.2 diluted 1:800) which were sterilized after filtration through a 0.22 filter.

The particle count data demonstrated the effectiveness of the AC-AM Fabric in reducing the particle (bacterial) count by 90% or more in 60 minutes, and the change in the particle size indicated damage to the bacterial cells. The reduction in viable bacteria was supported by standard plate count data. At 30 minutes the reduction of particle counts was 80-86% for saline, while in phosphate the reduction was 91-95%. This was also supported by viable bacteria counts.

Aerosol Test [37]

Test swatches were inoculated with an aerosol of the test bacteria produced in the Andersen Sampler used for bacterial barrier efficiency testing. This method provides a homogeneous distribution of innoculum over the entire surface of the swatch. Swatches for 0 time exposures were cut septically into small pieces immediately upon removal from aerosolization and allowed to drop into the Letheen Broth. For dwell intervals (1/2 through 3 hours) the inoculated swatches were transferred to closed humidity chambers, the humidity of which were maintained at 92% R.H. at 22 C using a saturated aqueous solution of Na_2HPO_4 in the chamber. Upon termination of a given dwell interval, the swatch was removed and cut aseptically into small pieces as described above for elution in Letheen Broth. The eluting interval in 50 ml. Letheen Broth was 10 minutes using a shake speed of 8.5 for the Wrist Action Shaker. The Letheen Broth eluant was then decanted into a sterile centrifuge tube (Clay Adams Dynac II) and centrifuged at 300 r.p.m. for 2 minutes to separate media linters from the suspension. One ml. of the supernatant was then cultured. One ml. of the eluting medium cleared of media linters was transferred aseptically to a sterile plate to which was added 18 to 20 ml. of tryptic soy agar containing 0.7 gm Asolectin and 5.0 ml.Tween 80/L. Tables X and XI list results using the above protocol. These tables show clearly the total control of the test organisms *P. aeruginosa* and *E. coli* within 15 minutes. Considering the dosage level of 1.37 x 106 and 1.3 x 106/swatch respectively, these results are outstanding.

Adaptation Study (Table XII)

It has been observed in our laboratory that many traditional leaching types of antimicrobial agents are susceptible to inductive or mutative adaptation. Adaptation is a phenomenon whereby a cell adjusts enzymatically (inductive) or genetically (mutational) to a toxicant in its environment. A study was undertaken with silanequat treated surfaces to determine the potential for adaptation of Gram(-) and Gram(+) organisms after contact exposure. No increase in adaptive potential was noted after five successive

exposures. This indicates an extremely low potential for adaptation.

Odor Test (Table XIII)

Many nonwoven fabrics are used where microbial odors are a significant nuisance. Our experience with the reduction of microbial odors on woven fabrics has been through laboratory and odor panel testing.[27] The extension of this work was done with nonwovens. Typical diaper constructions were treated and put in capped jars. *Proteus mirabilis* and a small amount of artificial urine nutrient were added. Ammonia measurements were taken using Gastec® tubes. Results show clearly the value of the silanequat treatment in the reduction of microbial odors.

Scanning Electron Microscopy (SEM)

Bacterial dilutions were placed on SEM stubs (experimentals) to check for the correct bacterial count for electron microscopy using a light microscope. The experimental stubs were prepared for electron microscopy by placing a drop of water containing dilute bacterial cultures, adding appropriate fibers, incubating at room temperature, drying under vacuum, and treating with carbon and gold. SEM photomicrographs were made using a Cambridge Scanning Electron Microscope. Silanequat treated Curex and Sontara were used in the studies. These experiments confirmed the antimicrobial action of the silanequat on *E. coli* and *S. aureus.* The encapsulated bacterium *K. pneumoniae* was also tested. The ability of the silanequat to exert its antimicrobial influence through the capsule was demonstrated.[38] The disruption of the bacterial cells normal morphology can be seen below in Figure 2**.**
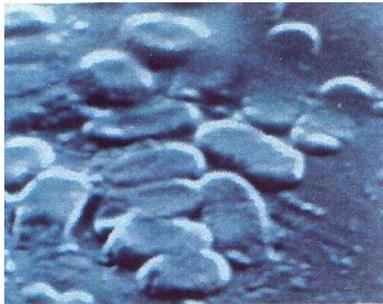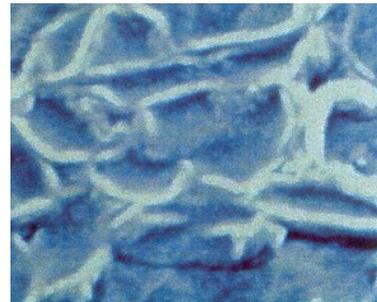

FIGURE 2: Before Treatment

FIGURE 2: After Treatment

Results - Scanning Electron Microscopy - ISO·BAC Fabric and Untreated Fabric
1. *Staphylococcus aureus*

Clinical Evaluations - In Vivo

Tests in a clinical environment are usually very complex because of the large number of uncontrollable variables. Yet, the final link to improvement in aseptic conditions is to be found in the clinical environment. Several studies are currently underway but only two of these will be reported on here.

Biobarrier Test [39]

An AC-AM Fabric instrument wrap was tested using a modified 28-day Shelf Life Test. Evaluations were conducted according to the method described by Schneider.[40] The test is referred to as a Simulated-Storage Evaluation in which the pathway between naturally occurring airborne bacteria and a nutrient media, supportive to their viability and proliferation, is blocked by the material in test. Two piles each of AC-AM Fabric Instrument Wrap, Kimguard, and repellent Sontara (non silanequat treated) and four piles each 140-count muslin (washed) were challenged in this test. Ten beakers, each containing sterile broth media that can support a broad class of microorganisms were covered with sterile packaging material as described above. The sterile covered containers were placed upright on a shelf in a storage room for 28 days to simulate in-use environmental exposure conditions. The test containers were not stressed by pressure from handling or stacking. Relative humidity ranged from 50% to 80%. The storage room was similar to a hospital storage room (in which sterile material is kept) in size, shelves, and placement of material

on shelves. Traffic into the storage room was not heavy, but was entered several times daily during the work week to remove or replace storage items. A failure was identified by visual observation of microbial growth as evidenced by turbidity. Confirmation of growth and organism types, or no growth, was done by slide preparation and subsequently determined by microscopic examination.

The results are tabulated in Table XIV. Several conclusions can be drawn from the data:

1. Linen afforded poor biobarrier protection as 60% of the containers showed growth during the test interval.

2. Kimguard afforded good protection as 90% of the test containers were negative.

3. AC-AM Fabric afforded the best protection. There were no failures.

4. Repellent Sontara (non silanequat treated) performed poorly as 50% of the test containers were contaminated.

The above data support the conclusion that the antimicrobial constituent in the AC-AM Fabric provides a substantial improvement in biobarrier activity over 140-count linen and Sontara. The interpretation of the Simulated-Storage Evaluation Test is direct. It is highly sensitive to detection of microbial penetration of the biobarrier by a broad class of microorganisms due to the moist environment and nutrient on the sterile side of the fabric. Since all classes of test material are exposed to identical test conditions, the observed differential penetration is a meaningful representation of the relative biobarrier of the four materials challenged. In this test, AC-AM Fabric Instrument Wrap had an equal chance of penetration by microbes, yet it provided an excellent biobarrier against contamination by environmental organisms.

Although these data were generated for CSR and instrument wraps interpolation to a variety of nonwovens and nonwoven environments is possible.

AC-AM Surgical Drape Reinforcement Study

Surgical Protocol: A double-blind study was conducted of 98 surgical cases using a reinforced laparotomy drape. The drape reinforcement was modified to consist of four sections (A,B,C, and D). Although all sections appeared to be identical, only two of the four were made of AC-AM Fabric. The location of the AC-AM Fabric sections were randomly varied. The surgical cases included clean, clean contaminated and contaminated cases.

Laboratory Protocol: After each procedure, viable bacteria from a portion of the AC-AM Fabric and non-treated reinforcement sections were removed. These swatches were agitated in a bacterial recovery solution and passed through a micro-porous filter. The filters were then placed on a pad containing nutrient media and incubated for 72 hours.

Clinical Results: Of the 98 surgical cases studied, this in-vivo study demonstrated that AC-AM Fabric reduces the number of viable potential pathogens in the critical areas by over 81%.

Comments from the study monitor include: "I would like to bring you up to date on the clinical project I have been involved in using the AC-AM Fabric surgical drape. The double-blind technique was used with random distribution of AC-AM Fabric and non-AC-AM Fabric strips on top of the surgical drape. Our early observations indicate a dramatic reduction in the bacterial colony count on the AC-AM Fabric versus the non-AC-AM Fabric strips. This held true with clean, clean contaminated, and contaminated surgical procedures of varying lengths of time. This data in the operating room certainly appears to verify the laboratory data done by American Convertors prior to release of the drapes for general use. The eduction in the number of bacterial colonies on the drape should contribute to a decreasing number of viable bacteria capable of infection. In addition, the mechanical usage of the drape has also been very satisfactory. The reinforced area in the AC-AM Fabric portion prevents strikethrough. The drape is soft and pliable enough to mold to the configuration of the patient. The surface also seems to prevent slippage of instruments." This study is still ongoing and will be the subject of a future publication.

Summary

The evolution of medical fabrics from the first tier usage of linen drapes to the second tier of

barrier and absorptive nonwovens has guided the way to the third tier nonwoven draping material - a safe, active nonwoven. This third tier nonwoven provides clearly demonstrable efficacy against a variety of laboratory and clinical (environmental) microorganisms. The microbiological test techniques used to demonstrate this effectiveness, as reported herein, are extremely varied. Results published here confirm the effectiveness of ISO•BAC Fabric under simulated and "real world" conditions. While we are still learning about the mechanism and performance of the ISO•BAC, we have confirmed that: (1) major levels of contamination are present at the surgical wound site in the area of the reinforcement around the fenestration, and (2) ISO•BAC (treated with the ÆGIS Microbe Shield) is capable of significantly reducing this level of contamination.

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TABLE I
Results: Minimum Inhibitory Concentration Test
Dow Corning 5700 Antimicrobial Agent

Test Organism	MIC (µg/ml)
Streptococcus Faecalis Gram (+) Bacteria	10
Escherichia coli Gram (-) Bacteria	100
Pseudomonas aeruginosa Gram (-) Bacteria	100
Aspergillus niger Fungus	1000

TABLE II
Results: American Association of Textile Chemists and Colorists
Method 30, Fungicides, Evaluation on Textiles
Dow Corning 5700 Antimicrobial Agent Treated Nonwovens

Percent of Sample Covered[1] After:

Sample	3 Days	5 Days	7 Days
Untreated	20	60	100
Treated Level A	0	5	20
Treated Level C	0	0	0

[1] *Aspergillus niger*

TABLE III
Results: American Association of Textile Chemists and Colorists
Method 100, Antimicrobials on Fabrics[1] - Dow Corning 5700 Antimicrobial Agent Treated Nonwovens

Sample	Microorganism	% Reduction
Control	Staphylococcus aureus	16
Treated[2]	Gram (+) bacteria	100
Control	*Escherichia coli*	0
Treated	Gram (-) Bacteria	99.6
Control	*Klebsiella pneumoniae*	0
Treated	Gram (-) Bacteria	100
Control	*Saccharomyces cerevisiae*	0
Treated	Yeast	99.9

[1] DuPont FC-170 surfactant used, substituted for Rohm and Haas Triton X-100

[2] Fabric was Kaycel

TABLE IV
Results: Clinical Isolate Control[2]
AEM 5700 Antimicrobial Treated Nonwovens

Sample	Microorganism	% Reduction
Untreated[1] Treated Inoculum	*Citerobacter diversus* Wound Isolate	14.3 93.6 0
Untreated Treated Inoculum	*Pseudomonas aeruginosa* Urine Isolate	28.3 99.9 0
Untreated Treated Inoculum	*Staphylococcus aureus* Wound Isolate	0 99.7 0
Untreated Treated Inoculum	*Escherichia coli* Urine Isolate	11.6 98.6 0
Untreated Treated Inoculum	*Proteus mirabilis* Wound Isolate	0 99.5 0

[1] Sontara Fabric
[2] Dow Corning CTM 0923

TABLE V
Results: Fluid Compatibility Tests
AEM 5700 Antimicrobial Treated ISO•BAC Fabric

Sample	Buffered Phosphate	Percent Reduction[1] with 15 Min. Contact	
		Saline	Serum
Untreated Linen	8	0	0
Untreated Sontara Nonwoven	0	0	0
Treated Sontara	99+	90+	90+

[1] Modified AATCC method 100 using test fluids *Klebsiella pneumonia* statistically significant at the 95% confidence level.

TABLE VI
Results: Surface Testing of Whole Blood and Bacteria[1]

Sample (Surface)	Contact Time (Minutes)	# of Organisms Per Ml	% Reduction
A) Green Surfical Linen	0	6,550	-
	1	4,750	27
	3	3,750	43
	5	400	94
	30	200	97
	60	200	97
	120	100	98
B) Non-Woven Tablecover From J&J Laparotomy Pack	0	22,100	-
	1	20,800	6
	3	15,300	31
	5	2,800	87
	30	550	98
	60	700	97
	120	200	99
C) HiLoft Untreated Control	0	10,450	-
	1	8,650	17
	3	8,900	15
	5	200	98
	30	0	100
	60	0	100
	120	0	100
D) HiLoft with AEM 5700 Antimicrobial – ISO•BAC Fabric	0	12,500	-
	1	5,000	60
	3	5,700	54
	5	0	100
	30	0	100
	60	0	100
	120	0	100

[1] Inoculum: 90% Whole Fresh Rabbit Blood Contaminated with *Klebsiella pneumoniae* ATCC 4352

TABLE VII
Results: Comparison of Contaminated Whole Blood Versus Defibrinated Blood in Solution and on Line

Test Method	Contact Time (Minutes)	Whole Blood	Defibrinated Blood
A) Solution Test (Organism Added to Blood)[1]	0	30,000	14,726
	1	30,000	15,375
	2	30,000	14,650
	3	2,990	13,900
	5	309	14,275
	30	-	15,125
	120	2	14,625
	360	-	13,375
B) Surface Test (Contaminated Blood Added to Linen Swatches)[2]	0	6,550	8,750
	1	4,750	9,300
	2	5,600	9,000
	3	3,750	8,700
	5	400	8,850
	30	200	9,900
	60	200	10,350
	120	100	10,850
	180	100	7,650

[1] Retrievals from the solutions.
[2] Modified AATCC-100 Padding Test, *Klebsiella pneumoniae* preinoculated into test fluids.

TABLE VIII Results: Surface Testing of Defibrinated Blood and Bacteria[1]			
Sample (Surface)	Contact Time (Minutes)	# of Organisms Per Ml	% Reduction
A) Green Surgical Linen	0	8,750	-
	1	9,300	0
	3	8,700	0
	5	8,850	0
	30	9,900	0
	60	10,350	0
	120	10,850	0
B) Non-Woven Tablecover From J&J Laparotomy Pack	0	14,050	-
	1	17,450	0
	3	13,750	2
	5	13,400	5
	30	15,350	0
	60	16,450	0
	120	17,800	0
C) HiLoft Untreated Control	0	13,650	-
	1	14,150	0
	3	13,600	0
	5	14,000	0
	30	13,750	0
	60	14,600	0
	180	16,850	0

D) HiLoft with AEM 5700
   Antimicrobial – ISO•BAC
    Fabric
	0	14,900	-

	1	15,400	0
	3	14,400	3
	5	12,400	17
	30	6,050	59
	60	5,650	62
	180	4,200	72

[1] Inoculum: 90% Whole Fresh Rabbit Blood Contaminated with *Klebsiella pneumoniae* ATCC 4352

TABLE IX Results: Preliminary Tests Comparing the Reduction in Count of *Staphylococcus epidermidis* Applied to HiLoft ISO•BAC Compared to Untreated Control						
Bacteria Suspended in	Count/0.1 ml $(x10^6)$	Swatch Wetted	Repl.	Count/ml $(x10^3)$		% Reduction
				Control	Treated	
Clark-Lubs KH_2PO_4	1.46	Clark-Lubs	1	198	0	100
			2	201	0	100
Acta Sweat	1.48	Acta Sweat	1	209	0	100
			2	214	0	100

TABLE X
Results: Aerosol Test ISO•BAC Control of *Pseudomonas aeruginosa* in Saline[1]

		Count/ml (x10^3 at Each Dwell Interval)					
Media No.	Repl.	0	¼ Hour	½ Hour	1 Hour	2 Hour	3 Hour
Control[2]	1	114	120	102	136	122	126
	2	115	101	91	107	112	114
	3	98	116	116	110	92	121
	Av.	109	112	103	118	109	120
ISO•BAC Level A	1	11	0	0	0	0	0
	2	16	0	0	0	0	0
	3	19	0	0	0	0	0
	Av.	15	0	0	0	0	0
ISO•BAC Level B	1	14	0	0	0	0	0
	2	12	0	0	0	0	0
	3	15	0	0	0	0	0
	Av.	14	0	0	0	0	0

[1] Initial population in broth 98 x 10^7/ml. Diluted 1:100 in saline and delivered 0.14 ml as an aerosol via Harvard Infusion Pump. Population deposited on swatch 1.37 x 10^6 cells/ml
[2] Whatman No. 40 filter paper

TABLE XI
Results: Aerosol Test ISO•BAC Control of *Escherichia coli* in Saline[1]

		Count/ml (x10^3 at Each Dwell Interval)					
Media No.	Repl.	0	¼ Hour	½ Hour	1 Hour	2 Hour	3 Hour
Control[2]	1	136	110	103	115	108	123
	2	114	119	92	122	102	107
	3	122	140	112	108	124	98
	Av.	124	123	102	115	111	109
ISO•BAC Level A	1	14	0	0	0	0	0
	2	22	0	0	0	0	0
	3	19	0	0	0	0	0
	Av.	18	0	0	0	0	0
ISO•BAC Level B	1	23	0	0	0	0	0
	2	16	0	0	0	0	0
	3	18	0	0	0	0	0
	Av.	22	0	0	0	0	0

[1] Initial population in broth 131 x 10^6/ml. Diluted 1:20 in saline and delivered 0.2 ml as an aerosol via Harvard Infusion Pump. 1.3 x 10^6 cells deposited on swatch.
[2] Whatman No. 40 filter paper

TABLE XII
Results: Bacterial Adaptation Studies - AEM 5700 Antimicrobial Agent Treated Fabrics

	Percent Reduction[1]									
	Klebsiella pneumoniae					*Staphylococcus aureus*				
Exposure[2]	1	2	3	4	5	1	2	3	4	5
Control	0	0	0	0	0	10	5	9	13	26
Treated	99+	99+	99	98	99+	99	98	96	99	99

[1] Dow Corning CTM 0923 Shake Flask Test
[2] Shake Flask Survivors were used for subsequent exposures

TABLE XIII
Results: ISO•BAC Nonwoven Fabric[1]

Sample	Total Accumulated Ammonia (PPM) Producted After:			
	2 Hours	4 Hours	6 Hours	8 Hours
Untreated	0	0.5	5.5	55.5
Treated Level A	0	0	2	28
Treated Level B	0	0	0.5	3.5

[1] Test Organism: Proteus mirabilis (Clinical) Inoculum: 1,000,000 CFU/ml

TABLE XIV
Results: ISO•BAC
Shelf Life Simulated-Storage Evaluation[1]

	Jar	ISO•BAC (2 Ply)	Linen (140 Count, 4 Ply)	Sontara (2 Ply)	Kimguard (2 Ply)
Bottom Shelf	1	-	+	-	-
	2	-	+	-	-
	3	-	-	-	-
Middle Shelf	4	-	+	+	-
	5	-	-	+	-
	6	-	-	-	-
	7	(Cornyebacterium sp only)[2]	+	+	+
Top Shelf	8	-	+	+	-
	9	-	+	+	-
	10	-	-	-	-

[1] Method described by Schneider
[2] Not a valid contaminant as noted by investigator.
      + = Microbial growth (variations of mold, yeast, Gram-positive, and Gram-negative microorganisms).
   - = No microorganisms observed

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