

ANTIMICROBIAL ADVANCED WOUND CARE DRESSING

WHS poster #142

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QuickMedTechnologies

Summary

QuickMed Technologies (QMT) has developed a patented process (NIMBUS™) that permanently attaches a quaternary ammonium-based polymeric microbicide agent onto a range of physical substrates. The process has been shown to provide effective protection against pathogenic bacteria (including *Staphylococcus aureus* and *epidermidis*, *Pseudomonas aeruginosa*, *MRSA*, *VRE*), viruses and fungi. Extraction testing and zone of inhibition testing show no leaching of the microbicide agent from the substrate. Cytotoxicity testing by rabbit eye and skin tests, as well as guinea pig dermal sensitization test demonstrates that the agents utilized are harmless to normal cells. NIMBUS™ dressings have been prepared on a range of substrates that include cellululosics (cotton, rayon) as well as more advanced wound dressing substrates such as foams, and superabsorbents.

Current commercially available antimicrobial dressings have clearly demonstrated improved healing on chronic wounds, and improve patient outcomes, but are generally based on leaching microbicides. High cost, and the possibility of patients developing resistance to leached agents such as silver or antibiotics, has made prophylactic use of these materials potentially problematic.

NIMBUS™ dressings represent a novel category of materials that can be economically and safely applied as prophylactics to prevent the progression of low-to-medium colonized wounds to infected wounds. The manner of bacterial control is non-leaching and based on cell-wall disruption – both these traits minimize the possibility of pathogens generating resistance. Since the treatment adds comparatively small incremental costs to the production of a normal untreated dressing, prophylactics use is sufficiently economical to enable overall savings while improving patient outcomes. Treated cotton gauze dressings have passed the full required suite of safety testing required by the FDA for devices subjected to prolonged exposure (1 – 30 days) on compromised skin surfaces. These dressings are currently under review by the FDA for 510(k) approval to allow them to go to market. This technology was recently profiled in TIME magazine (Vol 167, issue #12, 2006, p57), in an article that profiled Quick-Med scientist Greg Schultz as part of a series of technology innovators.

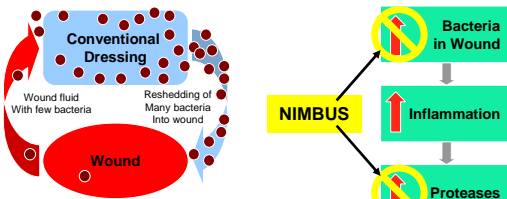


Figure 2. Reshedding of bacteria into a wound from a conventional dressing. Wound fluid absorbed by a non-antimicrobial dressing serves as nutrient to grow bacteria shed by the wound. The bacteria grown in the dressing can shed back into the wound to provide reinoculation.

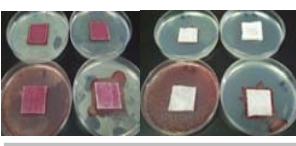


Figure 3. (above) Gauze sponge inoculated with various volumes of 5.8 x 10⁷ cfu/ml E. Coli in PBS (center-clockwise from top right: 0.5 ml, 0.75 ml, 2.0 ml, and 1.25 ml), then incubated for 15 h at 37°C on tryptic soy agar (Difco) containing 0.01 % TTC, and treated with a reactive dye (tetrazolium salt). The red color indicates areas of bacterial metabolism. Left panel shows untreated controls, right panel shows gauze treated by QMT's NIMBUS™ antimicrobial process. Note: there is no margin around the NIMBUS™ sponge, indicating that bactericidal activity is confined to the sponge itself, with no zone of inhibition.

Figure 1. Cascade of events initiated by the colonization of a wound by bacteria. The colonizing bacteria induce inflammation in the wound that, through the sequence of events depicted, results in decreased rate of healing for the wound. NIMBUS materials interrupt this sequence by controlling microbial growth, and by sequestering proteases. Reduced microbial populations will limit inflammatory response, and attendant protease production, thus helping to increase the rate of wound healing.

Bacteriocidal Efficacy Testing

Organism	% wound infection**	% killed	ATCC#
The most common wound-associated bacteria*			
<i>Staphylococcus aureus</i>	20%	>99.9999%	12600, 6538
<i>Staphylococcus epidermidis</i>	14%	>99.9999%	12228
<i>Enterococci spp</i>	12%	>99.9999%	19433
<i>Escherichia coli</i>	8%	>99.9999%	15597, 8739
<i>Pseudomonas aeruginosa</i>	8%	>99.9999%	51447, 15442, 9027
<i>Enterobacter spp</i>	7%	>99.9999%	13048
<i>Proteus spp</i>	3%	>99.9999%	13115
<i>Klebsiella pneumoniae</i>	3%	>99.9999%	13833
<i>Streptococci</i>	3%	>99.9999%	10096
<i>Candida albicans</i>	3%	>99.9999%	
Additional common bacterial species associated with [body] odor			
<i>Corynebacterium xerosis</i>		>99.9999%	7711
<i>Corynebacterium diptheriae</i>		>99.9999%	43145
<i>Micrococcus luteus</i>		>99.9999%	21102
<i>Protes vulgaris</i>		>99.9999%	13115
Additional common bacterial species associated with food contamination			
<i>Listeria monocytogenes</i>		>99.9999%	13932
<i>Salmonella choleraesuis</i>		>99.9999%	10708
Additional common bacterial species associated with burn wounds			
<i>Serratia marcescens</i>		>99.9999%	13880

*ATCC 10916, common bacterial species associated with wound infections

Table 2. Percentage of bacteria killed within the time indicated.

Time	Log Reduction of species, with ATCC numbers	SA(6538)	EC(15597)	PA(15442)	MRSA(BAA-4)	VRE(700221)
1 min	4.26	4.43	3.64	3.87	2.29	
10 min	3.71	4.29	3.95	4.22	3.32	
1 hr	4.83	5.23	5.30	5.45	5.89*	5.28
4 hrs	6.23*	6.72	6.41	6.83*	5.89*	5.63

*Standard deviation. Each measurement has an individual standard deviation. The numbers based on standard deviation in the growth rates. *Testing as per ASTM D-2915, using 10% FBS as hearse medium.

Antimicrobial Resistance

Resistant organisms are a growing concern in the modern health care environment. Resistant strains of particular concern are the antibiotic resistant strains MRSA, VRSA and VRE. Some UK healthcare professionals have recently adopted a designation of eMRSA for an epidemic form of MRSA, while public awareness of antibiotic resistant bacteria as a public health threat is increasing fueled by media reports on devastating nosocomial infections. Similarly, there have been increasing reports of resistance generated to silver by microbes. The antimicrobial properties of silver are attributed to its interruption of electron-transport and corruption of DNA replication mechanisms. Plasmid sequences encoding increased resistance to silver have been dated, resulting in some cases in bacteria with silver efflux pumping systems and increased synthesis of proteins that bind silver through sulfhydryl groups.⁷

There are two components to the generation of microbes that are resistant to certain agents: the mechanism of microbial control, and the local concentration of the antimicrobial. Both antibiotics and silver attack metabolic processes in microbes and corrupt replication, and various microbes have found ways to resist these processes. Quaternary ammonium compounds chemically destabilize the cell wall structures, inducing cellular collapse, as illustrated in figures 4 and 6. Since the chemistry of the cell wall is difficult for bacteria to alter, the generation of resistance to this mechanism is extremely unlikely. The second aspect of resistance generation is the local concentration of antimicrobial agent. Any agent that is used as a leachable will diffuse away from the dressing, until there is an area at a certain distance where the concentration of the agent falls below the lethal dose, or MIC. For a wound microbicide the concentration goes from lethal to zero at the physical boundary of the dressing, eliminating the "training zone" induced by leachable agents, as illustrated in figure 5. NIMBUS™ materials are unlikely to stimulate resistance in microbes since they are non-leachable, and rely on a cell wall disruption mechanism.

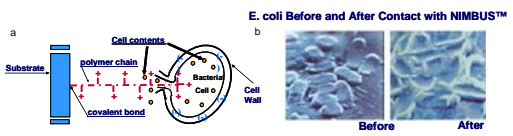


Figure 4: a and b. Mechanism of microbial activity of quaternary polymer. The image at left depicts the compromise of a bacterial cell wall by the NIMBUS polymer. The charged polymer chains compromise microbial cell walls, and induce cell lysis, as depicted in the before and after frames of E. coli bacteria that have had their cell walls compromised in the manner depicted, as can be seen from their appearance which resembles empty bags, or burst balloons.

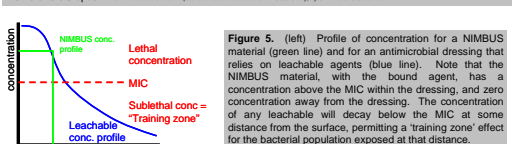


Figure 5. (left) Profile of concentration for a NIMBUS material (green line) and for an antimicrobial dressing that relies on leachable agents (blue line). Note that the NIMBUS material, with the bound agent, has a concentration above the MIC within the dressing, and zero concentration away from the dressing. The concentration of any leachable will decay below the MIC at some distance from the surface, permitting a "training zone" effect for the bacterial population exposed at that distance.

Conclusions

The benefits of antimicrobial wound dressings are too numerous to ignore. In many cases, dressings that might produce benefits for patients are not applied for economic reasons. Economics aside, the ubiquitous application of antimicrobial dressings in the face of even slight chances of resistance generation would be irresponsible. For these reasons, QMT designed NIMBUS technologies, which permit the treatment of various substrates including cotton, rayon, alginate and GCMs, for less than a cent per 4x4 dressing. Silver in powder or nanocrystalline form adds at least a magnitude more, typically more than \$4 per square inch. Since resistance generation is virtually implausible with NIMBUS, the prophylactic use of antimicrobial dressings can be realized by this technology; early application of such dressings in pre-clinical settings could prevent the progression of wounds otherwise likely to become chronic, and could be used in the prevention of nosocomial infections. NIMBUS materials are suggested as an efficient complement to the clinical use of leaching antimicrobials, with the goal of reducing the overall health care burden on society.

Table 3 (below). Reinoculation testing: performed by inoculation of 0.1 g gauze strip followed by overnight incubation in 10 % FBS, then reapplication of same process again.

Sample	Staph. aureus	E. coli
Control	4.1 x10 ⁶	3.2x10 ⁶
DC 5700 (Aegas)	1.6 x10 ⁶	2.2x10 ⁶
QMT NIMBUS®	1.7 x10 ⁶	2.7x10 ⁶

Table 4 (below) shows long term efficacy of antimicrobial activity, as measured in 10% FBS.

Sample	Staph. aureus (cfu/ml)	E. coli (cfu/ml)
48 hour control	<3 x10 ⁶	1.2 x10 ⁶
18 hour QMT sample	<3 (6 log kill)	<3 (6 log kill)
3 day control	4.3x10 ⁶	6.0 x10 ⁶
3 day QMT sample	<3 (6 log kill)	<3 (6 log kill)
7 day control	6.5 x10 ⁶	3.7 x10 ⁶
7 day QMT sample	<3 (6 log kill)	4.4 x10 ⁶ →4 log kill

NIMBUS embodiments

Materials substrates:
 Traditional wound dressings: Rayon, Cotton, Gauze
 Non-adherent dressings: hydrocolloid composites, superabsorbent polymer (SAP), biosynthesized cellulose, composites, hydrogel components, compression wraps

Physical embodiments:
 Medical (traditional): Conventional wound dressings based on gauze, nonwovens, etc.
 Medical (advanced): Advanced wound dressings based on foams and highly absorbent matrix materials
 Medical (nontraditional): Protective garments for secondary and tertiary bandaging (i.e. antimicrobial shirts for burn victims has been a particular request from clinical caregivers).
 Consumer textiles: Socks, T-shirts, various cotton and blended apparel items.

Current research directions:
 Protease inhibition with NIMBUS™ materials.

Treatment costs:
 The cost of materials associated with NIMBUS™ treatment is very small, particularly compared to expensive materials such as silver compounds. For many substrates the processing can be integrated readily into current manufacturing techniques, generating a significant added value for a small incremental cost.

NIMBUS™ Technology: Materials and Methods

NIMBUS™ is a family of technological processes that render substrates of choice antimicrobial. It is not a single chemical or a finished product in and of itself.

The NIMBUS™ process involves the permanent binding of a polymeric form of a quaternary ammonium based antimicrobial onto a surface. The details of the binding are specific to the substrate and application. The cationic polymer enables the binding of a second species for release if this is desired.

For medical grade applications the quality of binding is assessed by performing an extraction assay – where the substrate is incubated in saline at 70°C for 24 h or at 50°C for 72 h, and the extract is tested for antimicrobial activity against *Staphylococcus aureus*. Zone of inhibition (ZOI) experiments have also been conducted to demonstrate that no leachable agents are responsible for microbicial activity (see Figure 3).

Microbial assays on dressing material samples were performed using the AATCC method 100-1999 testing protocol, modified to more closely approximate anticipated use conditions (including the use of 10 % serum as medium). Briefly, swatches of material were inoculated with appropriate tiers of bacteria (typically 10⁶ to 10⁸ cfu/ml), extracted and grown on nutrient plates to enable comparison to control samples. This general protocol was followed for all testing involving swatches of substrate (such as woven cottons, dressing materials, etc.), with time points indicated where relevant, such as for re-inoculation testing, time to kill, or persistence of activity. While all results presented are not for identical substrates, the controls for each experiment were always untreated substrates of the same composition.

Animal Safety Testing

*Testing performed on NIMBUS™ treated cotton gauze, by Toxikon Laboratories, Bedford, MA
 NIMBUS materials have passed all standard toxicology tests for prolonged use materials (1-30 days) in direct contact with breached or compromised skin. The FDA is currently reviewing a 510(k) submission on the material.

- Safety tests performed and passed, as per ISO 10993.*
- Agar diffusion test
 - Intracutaneous Injection test
 - Kligman Maximization test (sensitization test)
 - Systemic Injection test

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