An Advanced Wound Dressing with Superabsorbent, Microbicidal and Haemostatic properties

Bernd Liesenfeld[1], Gregory Schultz[1,2], Christopher Batich[1,2], William Toreki[1], Roy Carr[1], David Lerner[1], Gerald Olderman[1,]; [1]Quick-Med Technologies, [2]University of Florida, [*]corresponding author*

Infection and Inflammation in Chronic Wounds

Optimal conventional treatments for chronic wounds are based on the concepts of wound bed preparation, which include elimination of necrotic tissue and fibrinous exudate, controlling infection, establishing moisture balance, and optimizing the epidermal margin.[1] Some chronic wounds fail to heal in a timely fashion in spite of the application of the principles of wound bed preparation. These wounds require treatment with advanced adjunct techniques, which include topical administration of growth factors,[2,3] bioengineered skin substitutes,[4] or surgical intervention for closure. Although advanced technologies have been proven effective in controlled clinical studies, they are expensive and are not universally available to all patients. There is a need for an inexpensive, readily available, simple therapy that can be added to the concept of wound bed preparation.

The central principles of wound bed preparation include controlling infection and inflammation. A wound is usually considered to be clinically infected if it harbors a bacterial burden that exceeds 10^5 cfu/g of tissue. Inflammatory responses, however, can be elicited from bacterial burdens that are significantly below the threshold criteria for being clinically infected or 'critically colonized'. Studies have characterized the molecular and cellular environments of chronic skin wounds (shown as a cascade of events in **Figure 1**).[5]

Chronic wounds typically contain increased levels of bacteria (that may or may not meet the standard for infection), which cause increased levels of pro-inflammatory cytokines and increased levels of proteases. These factors, in turn, degrade extracellular matrix (ECM) components, growth factors and receptors that are essential for healing.[6] These observations lead to the hypothesis that correcting these molecular abnormalities would promote healing of chronic wounds. Studies from Trengove and colleagues[5] support this hypothesis by demonstrating that elevated levels of pro-inflammatory cytokines and proteases decreased in chronic venous stasis ulcers as healing progressed. Additionally, Ladwig and colleagues[7] reported that the elevated ratios of matrix metalloproteinase-9 (MMP-9) to tissue inhibitor of metalloprotease-1 (TIMP-1) in wound fluids from pressure ulcer patients correlated with poor healing. Collectively, these clinical studies suggested that treatments that reduced the levels of bacteria, inflammatory cytokines, and proteases should improve healing of chronic wounds.

We hypothesize that many acute wounds in patients at risk for developing chronic wounds progress from initial acute wounds with low levels of bacteria to critically colonized or infected wounds because the bacteria growing in the wound fluid absorbed into common dressings (gauze, foams, alginates etc.) are shed back into the wound. The bacteria growing in the "reservoir" within these simple dressings re-inoculate the wound and promote progression (**Figures 1 and 2**) to critically colonized levels of bacteria.

We have developed what we call a Novel Intrinsically MicroBicidal Utility Substrate (NIMBUS®) process that permanently bonds a microbicidal quaternary polymer (a 'polyquat') to substrates such as cotton (gauze), cellulose (rayon) or synthetic polymers such as polyurethane, and have tested its microbicidal activity against a number of microbes that are important in wound healing.

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 is composed of the permanent binding of a polymeric form of 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 70º C for 24 h or at 50º C for 72 h, and the extract is tested for antimicrobial activity against *Staphylococcus aureus*. In most cases, zone of inhibition (ZOI) experiments were also conducted to demonstrate that no leachable agents were responsible for microbicidal activity (see **Figure 3** below).

Microbicidal assays on dressing material samples were performed using a suitably modified AATCC method 100 testing protocol. Briefly, swatches of material were inoculated with appropriate titers of bacteria (typically 10^6 cfu/ml, incubated overnight, 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.

Experiments demonstrating Zone of Inhibition (ZOI)

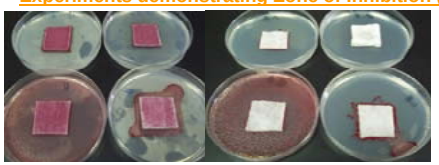

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Figure 3. Gauze sponge inoculated with various volumes of 5.8 x 10^3 cfu/ml *E. Coli* in PBS (counter-clockwise from top right: 0.5 ml, 0.75 ml, 2.0 ml, and 1.25 ml), then incubated for 15 h at 37ºC on tryptic soy agar (Difco) containing 0.01 % TTC, and treated with a reactive vital 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.

Bacteriocidal Efficacy and Time to Kill

Kill levels for:	Percent killed	ATCC #
Staphylococcus aureus	>99.9999%	12600, 6538
Staphylococcus epidirmidis	>99.9999%	12228
Escherichia coli	>99.9999%	15597, 8739
Klebsiella pneumoniae	>99.9999%	13833
Pseudomonas aeruginosa	>99.9999%	51447
Proteus vulgaris	>99.9999%	13115
Serratia marcescens	>99.9999%	13880
Enterococcus faecalis	>99.9999%	19433
Enterobacter aerogenes	>99.9999%	130483
Micrococcus luteus	>99.9995%	21102
Candida albicans	>99.9995%	
MRSA (methicillin resistant Staphylococcus aureus)	>99.999%	700221
VRE (vancomicin resistant Enterococcus faecium)	>99.999%	BAA-44
Bacteriophage MS-2 (an RNA virus)	>99.994%	
Bacteriophage PRD1 (a DNA virus)	>99.87%	

Table 1 (above). Tested in 10 % fetal bovine serum (fbs), using a modified AATCC method 100 protocol.

Table 2 (below) shows percentage of bacteria killed within time indicated;tested in 10 % fbs.

Time (min)	Staph. a.	E. coli
1	99.82 %	96.43%
10	99.95%	99.98%
20	99.95%	99.99%
30	99.993%	99.998%
60	99.992%	99.998%
4 hours	99.998%	99.9999%
8 hours	99.9999%	99.9999%
12 hours	99.9999%	99.9999%

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

Sample	Staph. aureus	E. coli
Control	4.1 x10^8	3.2x10^8
DC 5700 (Aegis)	1.6 x 10^6	2.2x10^8
QMT NIMBUS®	1.7 x 10^2	2.7x10^2

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

Sample	Staph. aureus (cfu/mL)	E. coli. (cfu/mL)
18 hour control	3.8 x10^6	1.2 x10^5
18 hour QMT sample	< 3 (6 log kill)	<3 (6 log kill)
3 day control	4.3x10^6	6.0 x10^5
3 day QMT sample	<3 (6 log kill)	<3 (6 log kill)
7 day control	6.5 x10^6	3.7 x10^5
7 day QMT sample	<3 (6 log kill)	4.4 x 10^2 –4 log kill

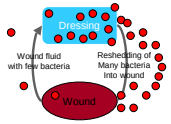


Figure 2. Reshedding of bacteria into wound. Wound fluid absorbed by the dressing serves as nutrient to grow bacteria shed by the wound. The bacteria grown in the dressing can shed back into the wound to provide reinoculation.

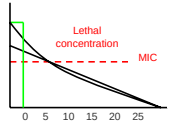


Figure 4. NIMBUS® interface. Nimbus® concentration gradient shown in green; black lines depict possible concentration gradients for leaching species.

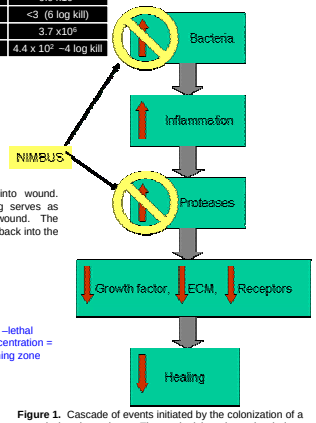


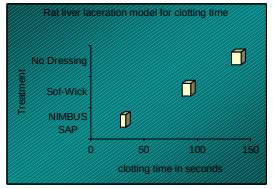
Figure 1. Cascade of events initiated by the colonization of a wound by bacteria. The colonizing bacteria induce inflammation in the wound that, through the sequence of events depicted, results in decreased rate of healing for the wound.

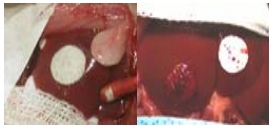
Haemostatic Testing of NIMBUS SAP formulation

Chart 1 (at right) and **Figure 5** (below to the right) show the haemostatic testing of a NIMBUS® processed superabsorbent polymer on a rat liver laceration model for clotting time.

The liver of an anesthetized rat was placed on a Teflon slide. A stab injury was made to the liver with a scalpel, and a 1 cm disk of the test dressing was placed on the wound. Bleeding time was measured in 15 s intervals.

The left panel shows the setup, while the right panel shows a post-experiment comparison of the NIMBUS® SAP sponge and a control. The cellulose sponge on the left is saturated while the NIMBUS® SAP sample on the right rapidly absorbs about 50x its weight in blood and stops the hemorrhage. The chart above shows data for experiments with no dressing, Sof-Wick dressing (J&J™) and NIMBUS® SAP, with bar thickness indicating one standard deviation.





Discussion

Wound Bed preparation emphasizes the need to reduce levels of inflammation and infection in wounds to promote healing. This has led to the development of several types of dressings that are approved as bacterial barriers. All these have the property that they release small microbicidal molecules, such as silver, iodine or biguanide, into wound fluid. Particularly the silver releasing dressings have seen significant growth in the marketplace and interest from researchers, clinicians and producers.

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. This is not lost on medical device producers seeking to provide more effective products for the patient that at the same time reduce risks – both present (infection today) and future (acquired resistances to important antibiotics or antimicrobials).

All antimicrobial dressings that are currently on the market are FDA cleared only as bacterial barriers. None are cleared for the treatment of clinically infected wounds. Anecdotally, there is evidence that modern silver releasing antimicrobial dressings have the capacity to aid the healing of chronic wounds that have proven slow to heal when treated with conventional dressings. However, a known risk of using small diffusible molecules such as silver is the development of bacterial resistance.

A good bacterial barrier is fundamental to the successful treatment of a wound – particularly chronic wounds that are slow to heal under the best of circumstances. A primary selling point for any bacterial barrier dressing is that it can protect from outside sources of infection gaining entry to the wound site. This function, however, is also provided by other physical barriers such as films at lower expense. A less obvious virtue of an antimicrobial dressing is that it will protect the wound from bacteria that are grown within a dressing shedding back into the wound (see diagram in **Figure 2**). A wound will exude fluids and bacteria into any absorbent dressing. Given only a short amount of time (days) a small inoculum of bacteria exuded from a wound that is not clinically infected will flourish in a conventional dressing, fed by a nutritious broth of wound fluids. During the normal course of patient movement, the compression of the dressing will, in sponge-like fashion, expel its contents in all directions – including back into the wound. This re-inoculation of the wound can help overwhelm the inflammatory defense of the wound, and provoke a clinical infection. The use of an antimicrobial dressing removes this source of re-inoculation by killing the bacteria as they enter the dressing. The lower bacterial burden that the wound will face under an antimicrobial dressing will contribute to a better clinical outcome in the healing of the wound. Any antimicrobial dressing that is effective at controlling bacterial growth within the dressing should therefore provide benefit in the outcome.

The novelty of the NIMBUS® process is that, unlike other antimicrobials that rely on the leaching of agents for their activity, the antimicrobial agent is permanently bonded to the substrate. This is demonstrated by the zone of inhibition testing shown in **Figure 3**. As shown in **Figure 4**, since the agent is bonded to the substrate, there is effectively a zero-length interface (shown by green line) as the concentration of antimicrobial agent goes from lethal (in the dressing) to zero (away from the dressing). Any design that relies upon leaching, particularly of small molecules, will have diffusion of the agent so that at some finite distance from the source there will be a concentration of said agent that is below the minimum inhibitory concentration (MIC), that will act as a "training zone". As bacteria continue to grow in this "training zone" a natural selection permits those acquiring survival traits to populate the upstream environment.

An additional component of acquired resistance is the mechanism of microbicidal activity, and this is where a further novel feature of NIMBUS® treated materials emerges. Antibiotic resistance is plasmid mediated – meaning that bacteria can transfer resistance plasmids to other bacteria. The increased use of silver compounds in recent years has yielded a commensurate increase in the reported incidences of silver resistant bacteria. 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 detailed, resulting in some cases in bacteria with silver efflux pumping systems and increased synthesis of proteins that bind silver through sulfhydryl groups.[8] The mechanism by which quaternary amine compounds effect their antimicrobial properties is through cell-wall disruption, which does not require complete entry into the cell by the compound. This type of mechanism is not as amenable to resistance generation, partly because the cell wall is difficult to remodel as a defense response. Out of all antimicrobial mechanisms, this is generally recognized as the least susceptible to acquired resistance.

NIMBUS compatible materials and development plan

The NIMBUS® family of processes has applications developed that are suitable for a wide variety of substrates. Below is a list of some applications that have been developed, and others that are currently in testing or in development.

Materials substrates:
- Traditional wound dressings: Rayon, Cotton, Gauze
- Advanced wound dressings. Polyurethane foam, hydrocolloid components, CMC superabsorbents, biosynthesized cellulose, composites, hydrogel components, compression wraps

Physical embodiments:
- Medical: Traditional and advanced wound dressings
- Consumer textiles: Socks, T-shirts…
- Microspheres for cosmetic and other applications

Current research directions:
- Protease inhibition using NIMBUS® materials
- Haemostatic testing for 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 minimal extra cost.

Animal and Clinical Testing Data

Rabbit eye irritation
- **Results: Non-irritating** (1)

Rabbit Skin Irritation
- **Results: Non-irritating** (2)

Guinea Pig Dermal Sensitization
- **Results: Non-sensitizing**(3)

(1) Protocol CL 1003
(2) Protocol CL 1005
(3) Protocol CL 1015

All testing conducted by: Geneva Test Labs, PO Box 140, Elkhorn, WI

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David Lerner - President (561) 750-4202
Email: dlerner@quickmedtech.com

Roy Carr – Director of Research & Development, Medical Devices (630) 756-1011
Email: rcarr@quickmedtech.com

Gerald M. Olderman - Ph.D. VP, Research & Development (781) 271-9893
Email: jolderman@quickmedtech.com