Infection and Inflammation in Chronic Wounds

Optimal conventional treatments for chronic wounds are based on the concept of wound bed preparation, which include elimination of necrotic tissue and bacterial intrusion; controlling infections, establishing moisture balance, and optimizing the epidermal margin.1 Some chronic wounds fail to heal in a timely fashion or fail to achieve the desired clinical outcomes. These patients require treatment with advanced adjunctive techniques, which cause topical side effects such as pain and hemostasis. Although these methods may offer initial clinical benefits, additional therapies are needed to avoid further complications and promote healing. Advanced wound dressings 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 has a bacterial burden that exceeds 10⁶ colony-forming units (CFUs) of tissue. Inflammatory responses, however, can be elicited from bacterial burdens that are significantly below the threshold criteria for being directionally infected or “clinically infected.” Studies have characterized this concept as a continuum of wound healing.2

Chronic wounds typically contain increased levels of bacteria, as many as 10⁶ to 10⁷ CFUs/g, and increased levels of proteases. These factors, in turn, degrade extracellular matrix (ECM) components, growth factors and receptors that are essential for healing. These observations lead to the hypothesis that these macromolecular wounds present priming healing of chronic wounds. Studies from Tempone and colleagues3 observed a correlation between increased bacterial load and delayed healing of chronic wounds.

We hypothesize that many chronic wounds are in a state of developing infected or infected wounds because the bacteria growing in the wound fluid absorbed into common dressing gauzes, bandages, or dressings, are shed back into the wound. The bacteria grow into the “reservoir.”4 Without an antimicrobial dressing to re-cleanse the wound and promote progression (Figures 1 and 2) and to chronically colonized levels of bacteria.

We have developed what we call a Novel Intrinsically MicroBicidal Utility Substrate (NIMBUS®) process that permanently binds a microbicidal quaternary ammonium (quaternary amine) to a substrate such as cotton, rayon or polyurethane membrane. We have tested its capabilities in both experimental and clinical environments with a microbicidal activity against a number of microbes that are important in wound healing.

NIMBUS® Technology: Materials and Methods

NIMBUS® is a family of technologies that render substrates of choice anticalmic. It is not a single chemical or a finished product in 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 polymeric process is that of a second or third cycle, whichever is desired. For medical grade applications the quality of bonding is assessed by performing an extraction assay where the substrate is immersed in water at 50 °C for 24 hrs (or 7 days) to simulate various biological environments and water exposure. The experiments were also conducted to demonstrate that no leachable agents were responsible for antimicrobial activity (aqueous phase 3). Microbials are on dressing material samples were performed using a modified ASTMD 2149-97 testing protocol. Briefly, monolayers of material were inoculated with appropriate forms of bacterial toxins (K. pneumoniae, Staph aureus, E. coli and P. aeruginosa) that represent the spectrum of bacterial enterotypes. The general procedure involves: a) loading the appropriate bacteria onto the treated and control media, b) growing cells for 24 hrs, c) removing cells, and d) counting the number of live cells from both treated and control media. The bacterial colonies are then enumerated according to the presence or absence of a color. Bacterial growth is interpreted as the presence or absence of a color. Bacterial growth is interpreted as the presence or absence of a colony.

Bacterial Efficacy and Time to Kill

<table>
<thead>
<tr>
<th>Bacterial Strain</th>
<th>Log Reduction</th>
<th>Time to Kill</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staph aureus</td>
<td>99.9999%</td>
<td>7 days</td>
</tr>
<tr>
<td>E. coli</td>
<td>99.9995%</td>
<td>3 days</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>99.998%</td>
<td>5 days</td>
</tr>
</tbody>
</table>

NIMBUS® interface.

Figure 1. Nimbus® concentration gradient across the training zone.

Figure 2. Sketching of bacteria into wound. The bacterial toxins are shed back into the market to grow bacteria shed by the wound. The bacteria grown in the dressing are shed back into the wound to provide inoculation.

Figure 3. Graph shows the initial inoculum of bacteria into the wound and the following severe to market to grow bacteria shed by the wound. The bacterial toxins are shed back into the wound to provide inoculation.

NIMBUS® compatible materials and development plan

The NIMBUS® family of processes has applications developed that are suitable for a wide variety of substrates that enable a broad range of applications, and others that are currently in testing or development.

Medical devices

- Autoclavable dressing: Biologics, Genes, Gauze
- Biopsy dressings: hydrogel dressings, hydrocolloid dressings, CMC superabsorbents, biosynthesized cellulose, composites, hydrogel components, compressed wraps

Physical applications

- Military and advanced wound dressings
- Consumer textiles: Socks, T-shirts...
- Microfibers for ointments and other applications

Current research directions

- Medical: Traditional and advanced wound dressings
- Consumer: Non-sensitizing

Animal and Clinical Testing Data

Rabbit skin irritation

- Result: Non-irritating

Hendrix skin irritation

- Result: Non-irritating

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Experiments demonstrating Zone of Inhibition (ZOI)

Figure 4. NIMBUS® interface after 7 days of incubation.

Table 1 shows the long term anti microbial activity, as measured in 10 day’s.

Table 1. Table 1 shows the long term anti microbial activity, as measured in 10 day’s.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Sample activity after 10 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>NIMBUS®</td>
<td>99.9999%</td>
</tr>
<tr>
<td>Control</td>
<td>99.999%</td>
</tr>
<tr>
<td>Conc.</td>
<td>X 10^6</td>
</tr>
</tbody>
</table>

References