Using the principle of hydrophobic interaction to bind and remove wound bacteria

Reducing the microbial load in an infected wound may help to promote healing. A hydrophobic dressing, which binds microbes whose surface contains water-repellent molecules, may reduce the use of antibiotics. This paper explains how

wound infection; hydrophobic; cell surface hydrophobicity

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kin, soft-tissue and wound infections are usually caused by wound pathogens such as Staphylococcus aureus and group A Streptococci (GAS).\(^1\) Pseudomonas aeruginosa, members of the Enterobacteriaceae, Enterococci, and other Streptococci families and anaerobic microbes such as Fusobacterium necrophorum and Bacteroides fragilis.\(^2\,3\)

Chronic infections are often of polymicrobial origin.\(^4\) In these, Trichophyton, and, subsequently, necrotising fasciitis, osteitis,\(^5,6,7\) skin.\(^15\) This can be mediated by receptor-specific the adhesion of the pathogenic microbe to damaged skin.\(^16\) This binding leads to adhesion to host tissue, which may lead to infection. Elgalai and Foster showed that over 85% of Staphylococcus aureus isolated from wound infections expressed binding of fibrinogen.\(^17\) Although isolates differed in their ability to bind plasma and ECM proteins, a significant correlation was found between expression of binding and infection of burns.

Several microbial cell surface structures have been reported to express hydrophobic properties, and are therefore likely to mediate adhesion to tissues by hydrophobic interaction.

Examples of hydrophobic tissue adhesions include:

- Fimbriae of Gram-negative bacteria\(^16\)
- Cell surface proteins of fungi\(^15,21\)
- S-layer proteins (capsule-like polysaccharide surface coatings)\(^22\)
- Lipoteichoic acid of Gram-positive bacteria\(^21\)
- Production of a carbohydrate polymer capsule — for example, by GAS and Staphylococcus aureus — renders the cell surface more hydrophilic (attracting water molecules),\(^1,2\) and therefore less prone to adhere to hydrophobic structures in human tissue or to hydrophobic dressings. This means that hydrophobic dressings are unlikely to be able to remove such bacteria.

Similarly, teichoic acid, a main constituent of the Staphylococcus aureus cell wall, confers a less negative charge on the bacterial cell surface, and mediates adhesion to various polymer surfaces.\(^24\) Thus, teichoic acid is less prone to mediate binding to tissue, other microbes or charged dressings by electrostatic interaction. Since they express lower cell surface hydrophobicity these microbes will also bind less avidly to hydrophobic dressings.

References

Protease production by microorganisms enhances the local spread of infection and tissue destruction. Matrix metalloproteinases (MMPs) interact with ECM proteins and enhance tissue invasion, MMP-13, a collagenase-3, impairs wound healing. MMP-19 regulates cellular growth factor response and inflammatory response by cleavage of cytokines and chemokines. MMP-19 is present in dermal fibroblasts and endothelial cells during wound repair, and it is postulated that it plays a role in angiogenesis.

Some extracellular toxins, like haemolysin, toxic shock syndrome toxin-1 (TSST-1), exfoliatin and superantigens of Staphylococcus aureus and GAS, contribute to tissue destruction and interfere with the immune defence system of these, the staphylococcal exfoliatin targets desmosomes, causing scalding of the epidermis, which may clinically correspond to second or third degree burns.

In experimental porcine wounds, Staphylococcus aureus and Pseudomonas aeruginosa form biofilms, which act as a barrier to antibiotic penetration and hamper signalling to the host immune system. This may be one cause of chronicity of wounds, and may be overlooked by wound-care strategies.

**Hydrophobic principle in bacteria removal**

When two water-repellent (hydrophobic) molecules collide with each other they increase the entropy (disorder of molecules). Although there is no force of attraction between the hydrophobic molecules, they will associate with each other by hydrophobic interaction and expel water molecules (Fig 1).

Microbes that express CSH during in vitro conditions that mimic a human wound are highly likely to bind to a hydrophobic dressing. Hydrophobic molecules may affect cell signalling and initiate innate immune responses. In Staphylococcus aureus, a conserved hydrophobic domain of the auto-inducing peptide binds to a hydrophobic pocket of the AgrC receptor, leading to activation of agr, which controls major virulence factors as well as quorum sensing. In this way the presence of CSH-expressing microbes in a wound may stimulate or antagonise wound healing.

This is an interesting area that so far has not been much explored.

**Expression of cell surface hydrophobicity by microbes**

Expression of CSH is an important mechanism of adhesion by microorganisms and is often a reaction to stress conditions such as starvation. CSH is mediated by cell surface proteins (hydrophobins).

Bacteria such as Peptostreptococci and other anaerobes express high CSH. However, strains of the same species may vary in their CSH. In Staphylococcus aureus, for example, staphylococcal delta-toxin, exfoliatin, TSST-1 and enterotoxin A are quite hydrophobic, whereas alpha-toxin and gamma-toxin are moderately hydrophobic, and the other staphylococcal enterotoxins have been shown to express low CSH. The expression of different toxins may thus influence the overall expression of CSH by an individual strain.

The expression of CSH is influenced by the availability of nutrients and the environmental atmosphere. In a previous study we grew microorganisms in a simulated wound environment comprising rich agar medium (haematin agar) covered by 1 mm human serum. Cultures were incubated in 5% CO₂ at 37°C. This resulted in expression of increased CSH compared with growth on poorer media incubated in air (Table 1).

The growth phase also influences CSH expression. Some bacteria form spores during starvation or other stress conditions. The spores of Bacillus subtilis express higher CSH than vegetative cells, and it is likely that this can be a more general property of bacterial spores.
In summary, the wound environment enhances expression of CSH by colonising microbes.23

Methods used to determine CSH include:23

- **Water contact angle**
- **Binding of aliphatic acids**
- **Adhesion to hydrocarbons**
- **Two-phase partitioning**
- **Hydrophobic interaction chromatography (HIC).**

In *vivo*, measuring of CSH by microbes provides information on whether or not they are likely to bind to a hydrophobic dressing *in vivo*.

## Binding of microorganisms

Cutisorb Sorbact (Abigo Medical AB, Askim, Sweden) is a hydrophobic coated dressing that uses the basic physicochemical principle of hydrophobic interaction to bind and subsequently remove microbes expressing CSH from wounds. In other words, only microbial cells expressing profound to moderate CSH, according to in *vivo* testing, will bind to the dressing; microbes expressing a hydrophilic cell surface will be left behind.

To study binding of microorganisms to a solid surface such as a wound dressing, we use bioluminescence to quantify the microbial ATP by referring to a species-specific standard curve. The binding to 1 square centimetre single layer of the Cutisorb Sorbact dressing was measured. Unlike conventional culture techniques, this method also quantifies adherent microbes.42

Using this method, binding of *Staphylococcus aureus* Newman and *Pseudomonas aeruginosa* BDS10 was measured from 0.5 minutes to 20 hours:

- **Binding increased after 10 minutes**
- **Binding reached a maximum at 120 minutes when 10^5 out of 10^9 added Psuedomonas aeruginosa had bound to the hydrophobic dressing**
- **Bacterial counts remained stable during 20 hours for *Pseudomonas aeruginosa*, and increased only from 10^4 to 10^6.5 after 20 hours for *Staphylococcus aureus*, showing that microbes multiply to a very low extent after binding to the hydrophobic dressing (data not shown).**

Adding increasing numbers of bacterial or fungal cells (10^4 to 10^8.5 bacterial cells and 10^2 to 10^5.5 fungal cells) showed that 10^4 cells of *Staphylococcus aureus* Newman bound and 10^4.8 cells of *Candida albicans* bound, but saturation (when more microbial cells could not bind to the dressing) was only shown for *Candida albicans*, where the curve tends to level off. When 10^10.3 cells of *Enterococcus faecalis* were added, 10^8 cells bound, again showing no saturation — in other words, still more bacteria could bind (data not shown). This means that the hydrophobic dressing is likely to be able to bind more than 10^6 *Staphylococcus aureus* and more than 10^7 *Enterococcus faecalis*. For *Bacteroides fragilis*, more than 10^6 cells bound out of the 10^8 added, and

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**Table 1. Influence of culture conditions on expression of cell surface hydrophobicity**

<table>
<thead>
<tr>
<th>Culture conditions</th>
<th><em>Staphylococcus aureus</em></th>
<th><em>Staphylococcus haemolyticus</em></th>
<th><em>Escherichia coli</em>**</th>
<th><em>Enterobacter cloacae</em></th>
<th><em>Pseudomonas aeruginosa</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood agar, air</td>
<td>&gt;2</td>
<td>0.25</td>
<td>&gt;2</td>
<td>&gt;2</td>
<td>&gt;2</td>
</tr>
<tr>
<td>Blood agar, 5% CO₂</td>
<td>2</td>
<td>0.1</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Blood plus serum, air</td>
<td>2</td>
<td>0.1</td>
<td>1</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Blood plus serum, 5% CO₂</td>
<td>1</td>
<td>0.01</td>
<td>0.5</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Blood plus inactivated serum, 5% CO₂</td>
<td>1</td>
<td>0.01</td>
<td>0.5</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Haematin agar, air</td>
<td>&gt;2</td>
<td>0.1</td>
<td>2</td>
<td>&gt;2</td>
<td>&gt;2</td>
</tr>
<tr>
<td>Haematin agar, 5% CO₂</td>
<td>2</td>
<td>0.1</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Haematin plus serum, air</td>
<td>1</td>
<td>0.01</td>
<td>0.5</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Haematin plus serum, 5% CO₂</td>
<td>0.5</td>
<td>0.01</td>
<td>0.25</td>
<td>0.5</td>
<td>1</td>
</tr>
<tr>
<td>Haematin plus inactivated serum, 5% CO₂</td>
<td>0.5</td>
<td>0.01</td>
<td>0.25</td>
<td>0.5</td>
<td>1</td>
</tr>
</tbody>
</table>

* Cell surface hydrophobicity was analysed by salt aggregation test (SAT). Results given are the lowest concentration of NH₄SO₃ giving visible aggregation. Two methicillin-resistant *Staphylococcus aureus* (MRSA) strains and four methicillin-sensitive *Staphylococcus aureus* strains were tested, giving the same results

**Notes:** Two *Escherichia coli* strains were tested, giving the same results

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If substances used in wound treatment decrease or abolish CSH, hydrophobic dressings become less effective. We therefore explored the influence of disinfectants, antiseptics and a cutaneous pain-relieving cream, lidocaine (Emla), on CSH expression. The substances used were:

- Octenidine dihydrochloride with phenoxethanol (Octenisept, Schülke & Mayr, Norderstedt, Germany); there is no UK equivalent
- 2-propanol, 1-propanol, 2-biphenylol (Kodan, Schülke & Mayr, Norderstedt, Germany); UK equivalents are Hibisol, Manusept, Mediswab, Sterets H
- Ethacridine lactate (Rivanal, Chinisol, Seelze, Germany); the UK equivalent, Burn Aid, is no longer available
- Povidone-iodine (Betadiona, Mundipharma GmbH, Limburg, Germany); UK equivalent is Betadine
- Hexamethylen biguanide (Lavasept, Fresenius Kabi, Bad Homburg, Germany); no UK equivalent
- Modified starch polymer with glycerol (Askinha hydrogel, B. Braun Hospicare, Collooney, Ireland)

Sodium chloride (Hypergel, Mölnlycke Health Care AB, Sweden); UK equivalents are Flowfusor, Irriclens, Irripod, Miniversol, Normalis, Stericlen, Steripod, Verso

Washed bacterial suspensions (10^9 cells) were incubated with the substance for 15 minutes at room temperature. CSH was measured before and after using the salt aggregation test. Of the substances studied, only Emla abolished expression of CSH. However, as expected, treatment with Askinha Hydrogel decreased expression of CSH (Table 2), and so should not be used before treatment with a hydrophobic dressing.

**Clinical studies**

Few studies investigating the hydrophobic dressing have been published. An open study involving 31
patients with 32 infected wounds\(^{40}\) (diabetic, arteriosclerotic, postoperative or post-traumatic leg ulcers, and ulcerated leukaemic infiltrates) and another study\(^ {47}\) comprising 12 patients with infected wounds (pressure ulcers, burns and diabetic wounds) that did not heal during conventional treatment (cleansing, compression bandaging of venous leg ulcers, systemic antibiotic treatment and mobilisation) have been published. Results of the former show that signs of infection disappeared during treatment with the hydrophobic dressing in 69% of patients and remained unchanged or deteriorated in 31%.\(^ {48}\) In the latter study the chronic wounds healed following part-skin transplantation and use of the hydrophobic dressing over a six- to seven-week period.\(^ {47}\)

In 1990 we undertook a study on three patients with chronic leg ulcers treated with the hydrophobic dressing and compression therapy for four weeks.\(^ {48}\) The dressing reduced the bacterial load and pus secretion in all three patients. Quantitative bacterial cultures were taken twice weekly, nurses performed cleansing rate and wound healing. However, a trained nurse was a satisfactory method of estimating cleansing rate and wound healing. However, due to the complications associated with antibiotics, particularly those with a broader spectrum, there is a need to develop non-antibiotic management strategies.

Table 2. Influence of wound treatments on cell surface hydrophobicity

<table>
<thead>
<tr>
<th>Microbe</th>
<th>Octenisept</th>
<th>Kodan</th>
<th>Rivanol</th>
<th>Betaisodona</th>
<th>Lavasept</th>
<th>Askina hydrogel</th>
<th>Hypergel</th>
<th>Emla</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus aureus</td>
<td>0.12</td>
<td>0.25</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>1</td>
<td>0.1</td>
<td>≥2</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>0.12</td>
<td>0.12</td>
<td>0.25</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>0.25</td>
<td>≥2</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>0.25</td>
<td>0.12</td>
<td>0.5</td>
<td>1</td>
<td>0.1</td>
<td>0.5</td>
<td>0.1</td>
<td>2</td>
</tr>
</tbody>
</table>

Washed microbial cells (10\(^7\) cells) were incubated with the substance for 15 minutes at room temperature.

\(^ {49}\) Salt aggregation test (SAT) values are presented as a numerical value of the lowest concentration of ammonium chloride in which visible aggregation occurs; therefore, the lower the value, the more pronounced the cell surface hydrophobicity. This was measured before and after incubation. The original SAT value was 0.5.

57.1%, \(p=0.028\) and a reduction in the number of treatment days (9 ± 2 versus 11 ± 2, \(p=0.041\)).

The control group (14 patients) was treated with mobilisation, appropriate nutrition, broad-spectrum antibiotics, topical iodine solution, collagenase and medicated plaster (an unspecified hydrocolloid). In the study group the hydrophobic dressing was used instead of the medicated plaster.

An important finding was that, in five patients who could not be given systemic antibiotic treatment because of renal impairment, treatment with the hydrophobic dressing achieved comparable results to those for systemic broad-spectrum antibiotics plus the hydrophobic dressing.

**Conclusion**

The pathogenesis of acute and chronic skin and wound infections is multifactorial and influenced by the immune and nutritional status of the patient, the underlying vascular disease, diabetes, smoking status and the virulence properties of the infectious agents. Reducing the microbial load is therefore a hallmark of treatment. However, due to the complications associated with antibiotics, particularly those with a broader spectrum, there is a need to develop non-antibiotic management strategies.

### Box 2. Summary of the main findings

**Microbes that express cell surface hydrophobicity (CSH) — that is, are water repellent — are likely to bind to a hydrophobic dressing**

*Cutisorb Sorbact*, a dressing with a hydrophobic coating, binds and removes from wounds microbes expressing CSH

**Use of the dressing may reduce the microbial load in a wound**

The hydrophobic dressing should be used in wounds with high and medium exudate levels as hydrophobic interaction is most effective in a moist wound environment.
Strategies as an alternative or even adjunct to a decreased antibiotic treatment.

A hydrophobic dressing is a non-allergic, non-toxic alternative for reducing the microbial load in open wounds without enhancing nosocomial spread, and can reduce the use of antibiotics. Hydrophobic microorganisms bind to the dressing, preferably in a humid environment, and are removed with it. They multiply to quite a low extent when absorbed in the dressing, and may not produce extracellular toxins and enzymes. Mechanisms of resistance to hydrophobic interaction have not been described.

We are currently comparing adhesion on the hydrophobic dressing of microbes grown in wound-like conditions with that for alginates and different dressings. Clinical studies are also under way comparing the hydrophobic dressing with different dressings. A silver-containing dressing may initially be superior, but it is only a matter of time before we see the emergence of resistance to silver among Gram-positive bacteria. Indeed, *Staphylococcus aureus* commonly express resistance to other metals, such as mercury.

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