

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

Skin, 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*.^{1,2}

Chronic infections are often of polymicrobial origin.³ In these, *Trichophyton*, *Candida albicans* and other fungi are commonly isolated, while the role of anaerobic species is often underestimated.^{4,5}

Infection may lead to local tissue degradation and, subsequently, necrotising fasciitis, osteitis^{6,7} and septicæmia. Clinicians should be aware that:

- Surgical-site infection can be dependent on the procedure and the anatomical location¹
- Burns have a high potential to become infected^{8,9}
- Patients with diabetes can develop lower extremity wound infections associated with vascular insufficiency and/or minor trauma¹⁰
- Exacerbation of atopic dermatitis or psoriasis is associated with colonisation by superantigen-producing *Staphylococcus aureus*^{11,12}
- Animal-bite wounds may become infected with *Pasteurella multocida* or *Capnocytophaga* spp.¹³
- Wounds exposed to sea water may become infected with *Aeromonas* and *Vibrio* spp.¹⁴

The initial event of a skin or wound infection is the adhesion of the pathogenic microbe to damaged skin.¹⁵ This can be mediated by receptor-specific hydrophobic or electrostatic interactions between the microbe and human tissue structures.

- Hydrophobic (lacking an affinity for water molecules) interactions take place when molecules expressing cell-surface hydrophobicity (CSH) come into contact with each other
- Electrostatic interactions occur when a microbe, generally expressing a negative net surface charge, comes into contact with a tissue molecule expressing a positive charge.

Microbes

Microbial cell surface proteins mediate binding to extracellular matrix (ECM) proteins — fibronectin, collagen, vitronectin, laminin — and plasma proteins, such as fibrinogen, by receptor-specific interaction.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 bacteria¹⁸
- Cell surface proteins of fungi^{19,21}
- S-layer proteins (capsule-like polysaccharide surface coatings)²²
- Lipoteichoic acid of Gram-positive bacteria.²³

Production of a carbohydrate polymer capsule — for example, by GAS and *Staphylococcus aureus* — renders the cell surface more hydrophilic (attracting water molecules),^{21,23} 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.

A. Ljungh, MD, PhD, Associate Professor;
N. Yanagisawa, MD, PhD;
T. Wadström, MD, PhD, Professor;
all at Division of Bacteriology, Department Laboratory Medicine, Lund University, Lund, Sweden.
Email: Asa.ljungh@med.lu.se

References

- 1 Bowler, P.G., Davies, B.J. The microbiology of acute and chronic wounds. *Wounds* 1999; 11: 2-8.
- 2 Davies, C.E., Hill, K.E., Wilson, M.J. et al. Use of 16S ribosomal DNA PCR and denaturing gradient gel electrophoresis for analysis of the microfloras of healing and nonhealing chronic venous leg ulcers. *J Clin Microbiol* 2004; 42: 3549-3557.
- 3 Howell-Jones, R.S., Wilson, M.J., Hill, K.E. et al. A review of the microbiology, antibiotic usage and resistance in chronic wounds. *J Antimicrob Chemother* 2005; 55: 143-149.
- 4 Faergemann, J. Atopic dermatitis and fungi. *Clin Microbiol Rev* 2002; 15: 545-563.
- 5 Stephens, P., Wall, I.B., Wilson, M.J. et al. Anaerobic cocci populating the deep tissues of chronic wounds impair cellular wound healing responses in vitro. *Br J Dermatol* 2003; 148: 456-466.

6 Lobmann, R., Schultz, G., Lehnert, H. Proteases and the diabetic foot syndrome: mechanisms and therapeutic implications. *Diabetes Care* 2005; 28: 461-471.

7 Eklund, A.M., Valtonen, M., Werkkala, K.A. Prophylaxis of sternal wound infections with gentamicin-collagen implant: randomized controlled study in cardiac surgery. *J Hosp Infect* 2005; 59: 108-112.

8 Vindenes, H., Bjerknes, R. Microbial colonization of large wounds. *Burns* 1995; 21: 575-579.

9 Ha, U., Jin, S. Expression of the soxR gene of *Pseudomonas aeruginosa* is inducible during infection of burn wounds in mice and is required to cause efficient bacteremia. *Infect Immun* 1999; 67: 5324-5331.

10 Davidson, J.K. (ed.). *Clinical Diabetes Mellitus: A problem-oriented approach*. Thieme, 2000.

11 Hoeger, P.H., Lenz, W., Boutonnier, A., Fournier, J.M. Staphylococcal skin colonization in children with atopic dermatitis. Prevalence, persistence and transmission of toxigenic and nontoxigenic strains. *J Infect Dis* 1992; 165: 1064-1068.

12 Zollner, T.M., Wichelhaus, T.A., Hartung, A. et al. Colonization with superantigen-producing *Staphylococcus aureus* is associated with increased severity of atopic dermatitis. *Clin Exp Allergy* 2000; 30: 994-1000.

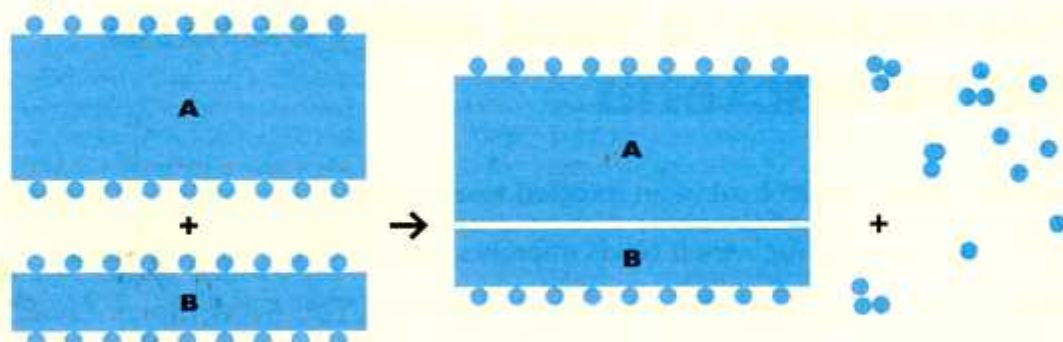
13 Bowler, P.G., Duerden, B.L., Armstrong, D.G. Wound microbiology and associated approaches to wound management. *Clin Microbiol Rev* 2001; 14: 244-269.

14 Tantilho, G.M., Fontanarosa, M., Di Pinto, A., Musti, M. Updated perspectives on emerging vibrios associated with human infections. *Lett Appl Microbiol* 2004; 39: 117-126.

15 Wadström, T., Eliasson, I., Holder, I., Ljungh, Å. (eds). *Pathogenesis of Wound and Biomaterial-associated Infections*. Springer-Verlag, 1990.

16 Ljungh, Å., Wadström, T. Binding of extracellular matrix proteins by microbes. In: Doyle R.J., Ofek, I. (eds). *Meth Enzymol - Microbial Adhesion*. Academic Press, 1995.

Fig 1. The hydrophobic principle: two hydrophobic molecules associate with each other and expel water



Reprinted with permission from *Journal of the Swedish Medical Association*⁴⁷

Protease production by microorganisms enhances the local spread of infection and tissue destruction.⁹ Matrix metalloproteases (MMPs) interact with ECM proteins and enhance tissue invasion.²⁵ MMP-13, a collagenase-3, impairs wound healing.²⁶ MMP-19 regulates cellular growth factor response and inflammatory response by cleavage of cytokines and chemokines.²⁷ 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.²⁸

In experimental porcine wounds, *Staphylococcus aureus* and *Pseudomonas aeruginosa* form biofilms^{29,30} 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).³¹ Although there is no force of attraction between the hydrophobic molecules, they will associate with each other by hydrophobic interaction and expel water molecules^{31,32} (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.³³ 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.³⁴ 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 microorganisms²³ and is often a reaction to stress conditions such as starvation. CSH is mediated by cell surface proteins (hydrophobins).³⁵ Bacteria such as Peptostreptococci and other anaerobes express high CSH.^{23,36-38} 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.³⁹ 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 1mm 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).⁴⁰

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,⁴¹ and it is likely that this can be a more general property of bacterial spores.

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

Culture conditions	<i>Staphylococcus aureus</i> *	<i>Staphylococcus haemolyticus</i>	<i>Escherichia coli</i> **	<i>Enterobacter cloacae</i>	<i>Pseudomonas aeruginosa</i>
Blood agar, air	>2	0.25	>2	>2	<2
Blood agar, 5% CO ₂	2	0.1	2	2	>2
Blood plus serum, air	2	0.1	1	2	2
Blood plus serum, 5% CO ₂	1	0.01	0.5	1	1
Blood plus inactivated serum, 5% CO ₂	1	0.01	0.5	1	1
Haematin agar, air	>2	0.1	2	>2	>2
Haematin agar, 5% CO ₂	2	0.1	2	2	2
Haematin plus serum, air	1	0.01	0.5	1	1
Haematin plus serum, 5% CO ₂	0.5	0.01	0.25	0.5	1
Haematin plus inactivated serum, 5% CO ₂	0.5	0.01	0.25	0.5	1

* Cell surface hydrophobicity was analysed by salt aggregation test (SAT). Results given are the lowest concentration of NH₄SO₄ giving visible aggregation. Two methicillin-resistant *Staphylococcus aureus* (MRSA) strains and four methicillin-sensitive *Staphylococcus aureus* strains were tested, giving the same results

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

In summary, the wound environment enhances expression of CSH by colonising microbes.⁴⁰

Methods used to determine CSH include:²¹

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

In vitro 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 vitro* 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* BD510 was measured from 0.5 minutes to 20 hours:

- Binding increased after 10 minutes
- Binding reached a maximum at 120 minutes when 10⁷ out of 10⁹ added *Pseudomonas aeruginosa* had bound to the hydrophobic dressing
- Bacterial counts remained stable during 20 hours for *Pseudomonas aeruginosa*, and increased only from 10⁶ 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 (10⁶ to 10^{9.5} bacterial cells and 10^{0.2} to 10^{7.5} fungal cells) showed that 10⁸ 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^{6.7} 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 10⁸ *Staphylococcus aureus* and more than 10^{6.7} *Enterococcus faecalis*. For *Bacteroides fragilis*, more than 10⁶ cells bound out of the 10⁹ added, and

17 Elgalal, I., Foster, H.A. Comparison of adhesion of wound isolates of *Staphylococcus aureus* to immobilized proteins. J Appl Microbiol 2003; 94: 3, 413-420.

18 Faris, A., Wadström, T., Freer, J.H. Hydrophobic adsorptive hemagglutinating properties of *Escherichia coli* possessing colonization factor antigens (CFA/I or CFA/II), type I pili, or other pili. Curr Microbiol 1981; 5: 67-72.

19 Singleton, D.R., Fidel Jr, P.L., Wozniak, K.L., Hazen, K.C. Contribution of cell surface hydrophobicity protein I (Csh1p) to virulence of hydrophobic *Candida albicans* serotype A cells. FEMS Microbiol Letts 2005; 244: 373-377.

20 Paris, S., Debeaupuis, J.-P., Cramer, R. et al. Conidial hydrophobins of *Aspergillus fumigatus*. Appl Environ Microbiol 2003; 69: 1581-1598.

21 Nakari-Setälä, T., Azeredo, J., Henriques, M. et al. Expression of a fungal hydrophobin in the *Saccharomyces cerevisiae* cell wall: effect on cell surface properties and immobilization. Appl Environ Microbiol 2002; 68: 3385-3391.

22 van der Mei, H.C., van de Belt-Gritter, B., Pouwels, P.H. et al. Cell surface hydrophobicity is conveyed by S-layer proteins: a study in recombinant lactobacilli. Coll Surf B: Biointerfaces 2003; 28: 127-134.

23 Doyle, R.J. Contribution of the hydrophobic effect to microbial adhesion. Microbes Infect 2000; 2: 391-400.

24 Gross, M., Cramton, S., Götz, F., Peschel, A. Key role of teichoic acid net charge in *Staphylococcus aureus* colonization of artificial surfaces. Infect Immun 2001; 69: 3423-3426.

25 Imper, V., von Wart, H.E. Substrate specificity and mechanism of substrate recognition of the matrix metalloproteinases. Academic Press, 1998.

26 Lapière, C.M. Collagenase and impaired wound healing. J Invest Dermatol 2003; 120: 12-13.

27 Hieta, N., Impola, U., López-Otin, C. et al. Matrix metalloproteinase-19 expression in dermal wounds and by fibroblasts in culture. *J Invest Dermatol* 2003; 121: 997-1004.

28 Persson, G., Norrgren, H., Hanson, C. et al. Staphylococcal epidermal necrolysis in a small child. Cases of Staphylococcus scalded skin syndrome in Sweden. *Läkartidningen, J Sw Med Assoc* 1999; 96: 1475-1476.

29 Akiyama, H., Huh, W.-K., Yamasaki, O. et al. Confocal laser scanning microscopic observation of glycocalyx production by Staphylococcus aureus in mouse skin: does S. aureus generally produce a biofilm on damaged skin? *Brit J Dermatol* 2002; 147: 879-885.

30 Serralta, V.W., Harrison-Balestra, C., Cozzaniga, A.L. et al. Lifestyles of bacteria in wounds: presence of biofilms? *Wounds* 2001; 13: 29-34.

31 Hjerten, S., Wadstrom, T. What types of bonds are responsible for the adhesion of bacteria and viruses to native and artificial surfaces? In: Wadstrom, T., Eliasson, I., Holder, I., Ljungh, A. (eds). *Pathogenesis of Wound and Biomaterial-associated Infections*. Springer Verlag, 1990.

32 Curtis, R.A., Steinbrecher, C., Heinemann, M. et al. Hydrophobic forces between protein molecules in aqueous solutions of concentrated electrolyte. *Biophys Chem* 2002; 98: 249-265.

33 Seong, S.-Y., Matzinger, P. Hydrophobicity: an ancient damage-associated molecular pattern that initiates innate immune responses. *Nature Rev Immunol* 2004; 4: 469-477.

34 Wright III, J.S., Lyon, G.J., George, E.A., Muir, T.W., Novick, R.P. Hydrophobic interactions drive ligand-receptor recognition for activation and inhibition of staphylococcal quorum sensing. *Proc Nat Acad Sci* 2004; 101: 16168-16173.

35 Wessels, J.G.H. Hydrophobins: proteins that change the nature of a fungal surface. *Adv Microbiol Physiol* 1997; 38: 1-45.



Fig 2. Cutisorb Sorbact incubated with a mixture of Staphylococcus aureus, Pseudomonas aeruginosa and Candida albicans. Microorganisms bind both to each other and to the dressing. (Raster electron microscopy)

for *Fusobacterium nucleatum*, $10^{7.3}$ cells bound out of the $10^{9.5}$ cells added.

Binding of a mixed culture containing *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans* to the hydrophobic dressing is shown in Fig 2. This figure also shows that, on the dressing, microbes coaggregate and bind to each other as well as to the dressing.

This dressing can be used on clinical infections because it reduces the microbial load in a wound without the use of antibiotics. *In vitro* testing and our studies in a simulated wound environment show that most wound pathogens are likely to express a higher CSH in wounds than in conventional *in vitro* culture. Reduction but not elimination of microbes in a wound may stimulate wound healing.⁴³

The dressing should be used on wounds with high and medium exudate levels as hydrophobic interaction is most effective in a moist environment. Furthermore, there is no risk of allergic reactions, and limited risk of spreading antibiotic-resistant microorganisms to the environment (Box 1).

The hydrophobic dressing is available in the UK, and recently has been included on the Drug Tariff.

Influence on the efficacy of the hydrophobic dressing *in vitro*

Wounds are commonly washed with disinfectants or antiseptics before dressing application.^{44,45} This may reduce expression of CSH by the microbes,⁴⁴ and therefore affect the action of wound dressings. Additionally, during wound debridement, pain relief is often necessary.

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 phenoxyethanol (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 (Rivanol, Chinosol, Seelze, Germany); the UK equivalent, Burn Aid, is no longer available
- Povidone-iodine (Betaisodona, Mundipharma GmbH, Limburg, Germany); UK equivalent is Betadine
- Hexamethylen biguanide (Lavasept, Fresenius Kabi, Bad Homburg, Germany); no UK equivalent
- Modified starch polymer with glycerol (Askina hydrogel, B. Braun Hospicare, Collooney, Ireland)
- Sodium chloride (Hypergel, Mölnlycke Health Care AB, Sweden); UK equivalents are Flowfusor, Irriclen, Irripod, Miniversol, Normasol, Stericlen, Steripod, Verso
- Lidocaine (Emla, AstraZeneca, London, UK).

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 Askina 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

Box 1. Properties of Cutisorb Sorbact

Binds microorganisms expressing cell surface hydrophobicity

Binds bacterial toxins

Leaves non-hydrophobic microorganisms in the wound to stimulate healing

Low likelihood of spreading bacteria during a dressing change

Non-allergenic

Optimal binding capacity in a moist environment

No development of antibiotic resistance

