

## Persistence of the chronic wound – implicating biofilm

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### Abstract

Chronic wounds by their very nature are recalcitrant and resistant to treatment. The pervading illness and pathology associated with the particular background disease, be it venous insufficiency, diabetes or the pathology underlying pressure ulcers have in the past been used as an explanation for non-healing and chronicity in these wounds. Thus managing poor perfusions, nutrition, sugar control, avoiding repetitive pressure have been and remain priorities in the overall treatment of these chronic wounds. It is apparent however, that in many cases, even when these processes are managed well, wounds still advance to non-healing and chronicity. Of late more and more authors are looking at biofilm formation and its behaviour characteristics as a possible explanation for chronicity in many wounds.<sup>1-13</sup>

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### The biofilm concept

Bacteria as we traditionally know them begin as single seeds of a (planktonic) bacterium. They express proteins and structures for motility (flagella) and attachment (fimbria). They aim to seed themselves and disperse to different areas thus exposing widespread areas to their presence and toxicity. In this form they are susceptible to antibiotics, some antiseptics and the immune system. In acute wounds they are usually rapidly destroyed or inactivated by neutrophils, antibodies and common wound bed preparations. They are also usually easily identified and cultured.

In the chronic wound however, the bacterium often takes on a different form. Small numbers of these single planktonic bacteria adhere to the surface of the wound by attaching to the exposed extracellular matrix; they multiply and develop over time into microcolonies. These colonies then aggregate into larger groups known as biofilms. The biofilm bacteria are encased in an extracellular polymeric matrix (EPS) which they manufacture themselves. Within 10 hours, each single-cell planktonic bacterium has differentiated into a complex community with defences and resistance to antibiotics.<sup>1,2,4,5,6,8</sup>

As the colony begins to grow signals are sent out amongst the cells – when the cells reach a certain density, known as a quorum (much the same as a minimum no of votes/people needed to pass a resolution in a meeting = a quorum) this density is sensed by the cells and they begin to elaborate virulence factors which are a potent defence against the body's polymorphonuclear leukocytes. This process is known as quorum sensing (QS)<sup>1,19</sup> (Figure 1).

Once the colonies of bacteria form a biofilm, individual bacteria can separate from the biofilm structure through a process called dispersion.

### Prevalence

There is little doubt about the existence of biofilm now. Biopsies of 50 chronic wounds and 16 acute wounds by James<sup>2</sup> showed that 60% of the chronic wound beds demonstrated definite biofilm. Of

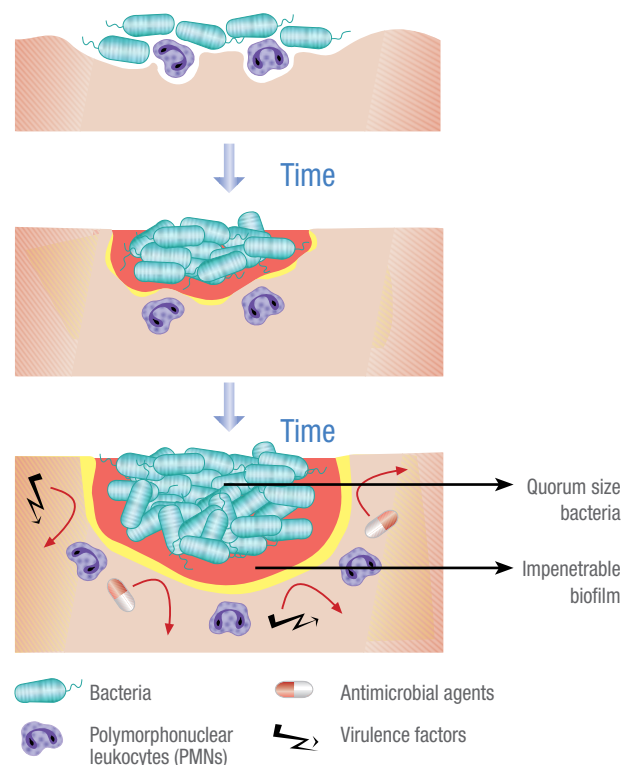


Figure 1: The mechanism of biofilm formation

the 16 acute wounds, only one showed a small patch of biofilm on the wound bed. Current estimates assume that 99.9% of the total microbial biomass on earth exists as a biofilm.<sup>2,5,11</sup> It has been estimated by the National Institutes of Health

(United States) that more than 80% of persistent bacterial infections are likely to involve biofilms.<sup>3,5,11</sup>

Specific areas where biofilms are known to be problematic have included hospital and portable water supplies, medical prosthetics and catheters, veterinary medicine, and cystic fibrosis.<sup>11</sup> Biofilms have commonly been identified on inert surfaces such as medical devices or on dead tissue such as sequestra of dead bone, but they can also form on living tissues, as in the case of endocarditis. Tissue samples have identified biofilm in patients with dental caries, periodontitis, otitis media, biliary tract infections, and bacterial prostatitis (i.e. non device-related chronic infections).<sup>3,5,11</sup>

One of the most commonly studied biofilm entities is oral biofilm. With progressive periodontal disease the patient is advised to brush and floss to remove their plaque and thus decrease the disease-causing biofilm. In the moist environment of the mouth, mature biofilm forms within 48 hours requiring brushing (debridement) at least twice a day.

A drier environment as in an ischaemic wound bed may not allow a biofilm to fully mature for 5–7 days (less frequent debridements needed).<sup>14</sup>

Among hospitalised patients, 8–10% are susceptible to infection by opportunistic pathogenic bacteria such as *P aeruginosa* and *S aureus*, which are notorious for forming chronic, biofilm-based infections in their hosts. Examining the chronic wound exudates and the *Pseudomonas* bacteria isolated typically from patients with cystic fibrosis, Bjarnsholdt et al found distinct microcolonies – the basal structures of bacterial biofilms.<sup>7</sup> They hypothesise that the lack of proper wound healing is at least in part caused by inefficient eradication of infecting, opportunistic pathogens. They suggest that the biofilm also offers a shielding mechanism from the phagocytic activity of the polymorphonuclear neutrophils (PMNs), the backbone of the immune response in patients with cystic fibrosis.<sup>7</sup>

Gjodsbol et al<sup>15</sup> investigated the bacterial profile of chronic venous leg ulcers and found *Staphylococcus aureus* (in 93.5% of the investigated ulcers), *Enterococcus faecalis* (71.7%), *Pseudomonas aeruginosa* (52.2%), coagulase-negative staphylococci (45.7%), *Proteus* species (41.3%), and anaerobic bacteria (39.1%). Bacterial infections are generally considered to be treatable by administration of antibiotics; however, this is not always true. Thus more than half of the chronic wounds investigated in this study were colonised with *P aeruginosa*. Furthermore, the *P aeruginosa*-infected wounds appeared significantly larger in terms of area than wounds that did not contain *P aeruginosa*.<sup>2,15</sup> The presence of *P aeruginosa* also seems to delay or even prevent the healing process – is this a biofilm phenomenon? Most authors seem convinced.

Biofilms continually release planktonic ‘seeds’ of bacteria from their biofilm matrix, which can bait the immune system to mount an inflammatory response. The biofilm can harvest nutrients from the host exudate that accompanies the inflammatory response. In this way, the sacrifice of a few bacteria promotes the survival of the community through continual nutrient acquisition. Suppressing the host response with steroids (?antiinflammatories) or physically removing the bacterial load through debridement may reduce the nutrients available to the bacteria.<sup>31</sup>

### Anti-microbial resistance

The EPS alluded to earlier offers structural stability and protection to the bacteria.<sup>5–9</sup> In this composite state, the bacteria resist the action of a variety of antimicrobial measures. The ability of *P aeruginosa* to form EPS-encapsulated biofilms is thought to be one of its main survival strategies in hostile environments. The EPS may contain polysaccharides, alginate (mucoid phenotype), extracellular

DNA, and other components such as proteins and lipids. Alginate enhances the three-dimensional (3D) structure of the biofilm,<sup>16</sup> acts as a scavenger of free oxygen radicals<sup>17</sup> prevents phagocytosis, and binds many cationic antibiotics such as aminoglycosides.<sup>18,19</sup>

### Identifying biofilm

As previously discussed biofilm bacteria are assembled in microcolonies and not evenly distributed within the wound. The implications are that cultures from a biopsy or swab are not likely to be representative for the total bacteriological load in the wound. The sample might be lacking the correct information of the colonising organisms.

Clinically, biofilms have been observed frequently as a translucent shiny/glazed manifestation on infected and nonhealing wound surfaces, often containing slough, not responding to traditional antimicrobial therapies and showing no signs of healing.<sup>11</sup>

### Biofilm models and treatment

A major concern in the management of nonhealing and infected wounds is the fact that bacteria within a biofilm phenotypically become more tolerant and resistant to antimicrobial therapies when compared with their planktonic counterparts.

A few publications have appeared specifically looking at isolating and treating biofilm colonies. A porcine model was used by Davis et al.<sup>3</sup> Using this model, partial thickness wounds were inoculated with a wound isolate *Staphylococcus aureus* strain. Wounds were then treated with either one of two topical antimicrobial agents (mupirocin cream or triple antibiotic ointment) within 15 minutes to target planktonic bacteria or 48 hours after initial inoculation to target biofilm-associated wound infection. Using light microscopy, scanning electron microscopy and epifluorescence microscopy, they were able to observe biofilm-like structures in wounds after 48 hours of inoculation and occlusion. Both mupirocin cream and the triple antibiotic ointment were effective in reducing planktonic *S aureus* but had reduced efficacy against biofilm-embedded *S aureus*. They demonstrated that *S aureus* formed firmly attached microcolonies and colonies of bacteria encased in an extracellular matrix on the surface of the wounds. These biofilm-like communities also demonstrated increased antimicrobial resistance when compared with their planktonic phenotype in vivo.<sup>3</sup>

A second study using a LabTekt slide model was used by Percival et al<sup>4</sup> in an effort to determine the antimicrobial efficacy of a silver-containing Hydrofiber® (SCH) dressing on bacteria growing in a biofilm state. The efficacy of silver dressings against biofilm had not been previously demonstrated. As antimicrobial dressings containing ionic silver are increasingly being used to help manage the microbial bioburden in infected or potentially infected chronic wounds, it is desirable that they are effective in preventing and breaking down biofilm formation, as microbes prefer to exist in a biofilm. This study showed that all strains of bacteria utilised readily formed biofilms after only 3 hours growth in the LabTekt slide model. Following real-time long-term visualisation studies (up to 72 hours), the SCH dressing was found to be effective in killing the tested bacteria. For bacteria growing as a biofilm phenotype, kill did not begin until **after** three hours. Over 90% kill was, however, achieved after a 24-hour contact time with the SCH dressing. The results showed that SCH dressings are effective at inhibiting certain biofilms and killing certain bacteria within a biofilm. The results also showed that the LabTekt biofilm model proved effective as a test for potential biofilm treatments in vitro.

Concurrent with this study was a recent study undertaken by Chaw et al<sup>20</sup> who demonstrated that silver ions have the ability to break up and disrupt biofilm structures at concentrations above 50 ppb.

The results generated in the study by Newman et al.<sup>21</sup> also suggest that *S aureus* is less susceptible to ionic silver than *P aeruginosa* – longer silver exposure time was required to induce cell death in *S aureus* (24 hours) compared to *P aeruginosa*.

Ideally, agents used in biofilm treatment should be able to disrupt its structure. Traditional antibiotics are better at destroying individual bacteria than colonies as seen in biofilm. Many agents are currently being investigated for use against biofilm – these agents mark a shift in traditional antibiotic mechanistic killing of bacteria. Rather they interfere with the formation of biofilm (xylitol, dispersin B, gallium), the attachment to the matrix (iron scavengers deferoxamine, lactoferrin, ethylene diamine tetraacetic acid [EDTA]) degrade EPS (dispersin B, alginate, phage depolymerases) or inhibit the QS virulence producing mechanism described above (RIP – RNA III inhibiting peptide; furanone C30).<sup>11</sup>

Strict adherence to wound management (especially repeated and adequate debridement) should not be underestimated in overcoming biofilm infestation. These principles together with local agents (lactoferrin and xylitol) were successfully used in patients with critical limb ischaemia (CLI). In a report of their results Wolcott and Roads<sup>11</sup> showed an impressive 77% rate of healing in these difficult-to-heal groups of patients. They attributed their excellent results to strict adherence to principles of wound healing (debridement, offloading, perfusion etc) causing a decrease in matrix metalloproteinases (MMPs) and elastase and decreased exudates in the wound environment. Secondly, by targeting biofilm specifically, they felt the effects of their antibiotic and hyperbaric oxygen (HBO) therapies were markedly improved.<sup>11</sup>

### The hypothesis

It would appear that more and more investigators are convinced that chronicity of a wound may be related in a large part to the presence of biofilm. The eradication of this virulent phenotype of bacterium is thus becoming an imperative in the treatment of wounds. Multiple authors<sup>22–27</sup> have looked at biofilm in the context of patients with cystic fibrosis (CF). They point out the obvious similarities with respect to the bacterial infection found in CF and chronic wound patients. They propose that the conditions are kept chronic by the bacterial burden especially that related to *P aeruginosa*, a common pathogen in CF. They propose that presence of this bacterium in the form of a biofilm and its excretion of damaging virulence factors including an efficient PMN shield, encourages bacterial persistence and may explain the extreme tolerance to antibiotics and the diminished capacity of the immune defence.

This view, in relation to chronic wound healing, is one now shared by a large number of wound care researchers.

### Future work and perspectives

Successful treatment of a non-healing wound depends on identifying and treating factors that impede the healing process. Until now, it has not been fully explained why chronic wounds have elevated MMPs or why PMNs exist in vast numbers in the chronic wound. It now seems apparent that the chronicity of the wound as manifested by the biochemistry just described may be related to the formation of biofilm. Our thinking about bacteria and its causation in infection needs to change – no longer can bacteria be considered unicellular independent pathogens, rather it is now important to understand

that they form multicellular tissue-like structures with major virulent capacity and antibiotic resistance.

Treatment scenarios are within reach. Lately, a number of studies have proven the concept of QS inhibitors (QSIs) as a relevant antimicrobial treatment.<sup>27–30</sup> Effective QS blockers have been designed, but further clinical tests including their relevance in wound-healing therapy are required.

In this regard Bjarnsholt et al conclude: “It is now generally accepted that all wounds are colonised; however, we believe that it is unrealistic to keep the wound sterile. By the use of QSI-based drugs, we propose that it might be possible to affect the wound to such an extent that the host itself is able to eliminate the infecting bacteria and recreate the normal healing process”.<sup>7</sup>

By intervening in the process of biofilm formation, we are once again facilitating the body's capacity to heal and tilting the odds toward an acute wound milieu with adequate defences for healing.

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## Cutimed® Sorbact® for colonised and infected wounds

Cutimed® Sorbact® makes use of hydrophobic interaction to remove pathogenic wound bacteria.

Hydrophobic interaction is a basic physical principle: hydrophobic (water-repellent) particles accumulate in an aqueous environment and are held together by the forces of the surrounding water molecules. Bacteria have certain hydrophobic cell surface structures. These are needed, for example, to adhere to host (wound) tissue in the initial phase of a wound infection. Cutimed Sorbact, on the other hand, is coated with a fatty acid derivative (**DACC**) which gives the dressing its strongly hydrophobic properties.

### The Sorbact method.

**Wound bacteria** are irreversibly bound to the dressing when they come into contact with the hydrophobic dressing fibres in the moist wound environment. Studies show that pathogenic wound bacteria have hydrophobic properties. And the more pathogenic they are, the more hydrophobic they are as well. Cutimed Sorbact utilises this principle. The more harmful the bacteria are, the more effective Cutimed Sorbact is. The **Sorbact method** also works effectively against fungi such as *Candida albicans*, so Cutimed Sorbact additionally offers a genuine alternative in the treatment of **dermal fungal infections**.

### Effective even against MRSA and VRE.

Fighting multi-resistant bacteria has become increasingly important in wound treatment and hospital management. It is good to know that the Sorbact method is also effective against MRSA (methicillin-resistant *Staph. aureus*) and VRE (vancomycin-resistant *Enterococcus*) as their resistance against antibiotics does not change their hydrophobic properties nor their ability to bind to Cutimed Sorbact.

Once bound, bacteria are **inactivated** and their metabolism is slowed down. As a result, replication is minimised and so is the formation of bacterial toxins that impair wound healing. An additional advantage of Cutimed Sorbact over chemically active agents such as silver is that the pathogens are effectively bound but not destroyed which prevents the release of bacterial endotoxins.

### Cutimed Sorbact gel

The new ready-to-use hydrogel impregnated dressing combines the antimicrobial Sorbact method with moist wound management. The hydrogel impregnation is free of preservatives and promotes autolytic debridement in sloughy or partly necrotic wounds.

### Advantages over other antimicrobial approaches.

✓	No development of bacterial or fungal resistances	that might be linked with the use of antiseptics or antibiotics as hydrophobic interaction is essential for microbial life.
✓	No risk of allergies	with Cutimed Sorbact dressing pads, swabs and ribbon gauze as the components are highly skin compatible.
✓	No cytotoxicity	as Cutimed Sorbact does not release any chemically active substances into the wound that might impair the healing process.
✓	No promotion of bacterial endotoxin release	because, unlike e.g. silver products, Cutimed Sorbact does not destroy bacteria. Thus <b>endotoxin release</b> from dead cells is prevented, and the natural wound healing is supported.
✓	No contraindications	with Cutimed Sorbact dressing pads, swabs and ribbon gauze: All products can be used without hesitation during pregnancy and breastfeeding as well as on children.