

WHAT IS THE ROLE OF BIOFILMS IN WOUND HEALING?

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VJ: 'It is debatable whether you can see a biofilm with the naked eye, as this normally requires the aid of a microscope'

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While biofilms have been known to microbiologists for a long time, the wound care community has only been aware of them for about 10 years. The discovery is generally attributed to Serralta et al (2001), however, the seminal article is perhaps that by Mertz et al (1987) who alluded to these structures without actually mentioning them by name.

A recent literature search in PubMed revealed over 18,000 articles mentioning biofilms since 1981. During this time a number of research articles have been published on biofilm structures in acute and chronic wounds, organisms responsible, and treatment strategies, the various conclusions being extrapolated largely from in vitro studies.

Our understanding is, in my opinion, still at an early stage. There is evidence that some acute wounds are likely to have biofilm colonies, as do a greater proportion of so-called chronic wounds. However, there is no evidence, or plausible suggestion, that all chronic wounds have biofilms. Equally, there is evidence that some commonly used topical antimicrobials will disrupt established biofilms in vitro. Among these are silver and honey.

Perhaps most important in a clinical sense is the evidence that supports debridement as a means of disrupting and/or removing superficial biofilms. This has become a contentious issue in recent years as clinical practice varies from 'aggressive' sharp methods to more 'gentle' passive autolytic methods. Which suits each wound and each patient? We await clarification.

It is always important to remember that, in some cases, biofilms can occupy deep tissues and may not always be disturbed by debridement of the

superficial tissue. This is important when clinicians observe wounds and assess the various 'chronicity factors' — all that glistens is not necessarily biofilm!

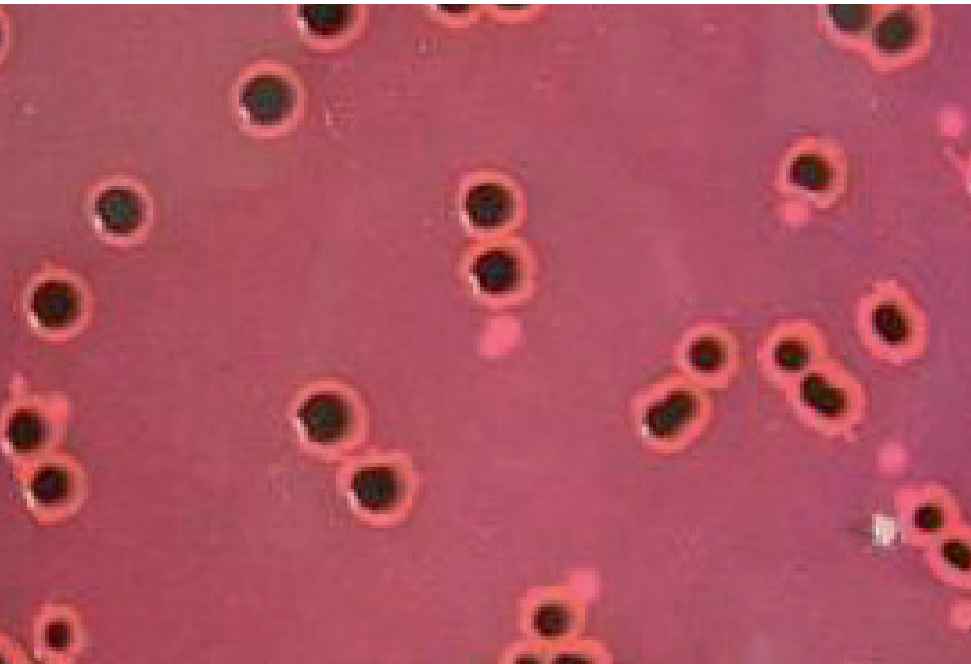
While biofilms may manifest as visible structures in other tissues, for example dental plaque (Marsh et al, 1995; 2011; Lovegrove, 2004), it is important to recognise that different organisms and different substrates are involved, making cross-referencing fraught with scientific inaccuracies (we need much more evidence to support clinical practice).

This debate is but a small part of the greater public discussion that is required. We need facts, not fiction or conjecture.

As an integral part of the *Wounds UK* journal debate programme, two acknowledged experts, Rose Cooper and Val Edwards-Jones, have been asked to address some of the most urgent questions on biofilms in wounds.
Richard White

What is your understanding of a biofilm?

RC: In natural environments most microbial cells grow within complex communities known as biofilms. Biofilms tend to form at junctions between solids, liquids or gases where available nutrients have accumulated. Biofilm formation involves the adherence of free-living (planktonic) pioneer species, followed by growth, division and microcolony formation. Other species may join at any time. As the microbial aggregate increases in size, individual cells produce chemical signals that allow the number of similar cells to be estimated. This form of cellular communication is known as quorum sensing — some signals allow cells of the same species



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to interact, and others facilitate inter-species communication. When a critical population size for each species is reached, the respective quorum sensing system influences the expression of genes and allows the aggregate to differentiate into a mature biofilm. In a mature biofilm microbial cells are encased within a sticky polymeric matrix that was synthesised collectively. It contains polysaccharides, nucleic acids and proteins and is known as an extracellular polymeric substance (EPS). Biofilm constituent cells usually have increased expression of virulence genes, but decreased growth rates. Since most antimicrobial agents interfere with biosynthetic pathways in actively growing cells, microbes within mature biofilms exhibit markedly decreased susceptibility to inhibitors. Fragments of biofilms can be shed and individual cells can revert to planktonic form to be disseminated to new sites.

VJ: A biofilm is a complex aggregate of microorganisms and an extracellular matrix of polymeric substances (e.g. polysaccharides) firmly attached to a surface. The microorganisms in the biofilm act as a community, each cell communicating with its neighbours through secreted signalling molecules. The extracellular matrix can shield the microorganisms from the outside

environment and protect them against the host's immune mechanisms and antimicrobial treatments.

What kind of wounds do you think are most affected

RC: In a landmark study (James et al, 2008), it was demonstrated by scanning electron microscopy and confocal scanning laser microscopy that biopsies collected from 1/16 acute wounds possessed a biofilm — in chronic wounds the figure was 30/50. Although chronicity was, therefore, associated with the presence of a biofilm ($p < 0.001$), failure to heal can be attributed to host factors, such as nutritional status, co-morbidities or concurrent therapies, and it is unsafe to assume that all chronic wounds possess biofilms. Moreover, biofilms of *Staphylococcus epidermidis* were discovered on sutures and staples removed from healed wounds, so we must not assume that the presence of a biofilm necessarily prevents healing (Gristina et al, 1985).

VJ: Chronic wounds are mostly affected. Following any trauma, a wound should heal within approximately two weeks unless there are co-morbidities that impede the wound healing mechanism. During the healing period, wounds often become contaminated and colonised with endogenous and

exogenous microorganisms. This does not always prevent the wound healing process unless the microorganisms produce virulence factors that can inhibit growth factors, other biological molecules or destroy newly formed tissue through toxin or enzyme production. In this case, the host response will elicit the classic signs of infection. If the microorganism does not produce virulence factors, then it is possible that the wound will become chronic, with the microorganisms forming a biofilm.

Can you see a biofilm?

RC: The resolution limit of the human eye depends on the individual, but it is thought to be about 0.25mm or 250µm, therefore, it is physically impossible to see anything smaller than this without some kind of enhancement. Many bacterial cells are 1–3µm in length, therefore, in order to observe individual cells in the laboratory a culture must be stained and viewed using a light microscope with 1,000 times magnification. When bacteria are cultivated on a plate culture, only colonies that contain at least 1,000,000 cells can be seen with the naked eye. Even though a biofilm contains millions of cells, it is not possible to discern a biofilm structure by the naked eye and the presence of slime or slough in a wound is not accepted as evidence of biofilm. Many bacteria can produce slimy or mucoid colonies on culture plates, but they do not form biofilms under these artificial conditions, so it is clear that appearances can be deceptive.

At the present time, there is no simple way to detect biofilms in wounds. When routine culturing methods were utilised to investigate the microbial flora of chronic wounds in 22 patients, only *Staphylococcus aureus* was detected in most wounds. Yet when the same samples were examined by immunofluorescent microscopy, microcolonies of *Pseudomonas aeruginosa* embedded in matrices were observed in the majority of wounds (Kirketerp-Møller et al, 2008). Using a similar

experimental approach, it is evident that there is non-random distribution of these two bacteria in chronic wounds, with *P. aeruginosa* at significantly further distances from the wound surface than *S. aureus* (Fazli et al, 2009). Therefore, biofilms are not exclusively associated with the wound surface.

VJ: It is debatable whether you can see a biofilm with the naked eye, as this normally requires the aid of a microscope. However, there may be subtle signs, which indicate that there is something adhering to the surface of the wound. For example, one type of biofilm is the slimy layer on the inside of a fish tank and it is possible to feel this layer or perhaps see light reflected from it. It is also possible to visualise a common biofilm — dental plaque — using a coloured disclosing fluid. It is important that biofilms are not confused with pseudomembranes, which can be physically removed from a surface. A pseudomembrane is a thin, adherent, grey-white exudative layer composed of necrotic epithelium and debris, fibrin, bacteria and neutrophils.

What effect can a biofilm have on the wound?

RC: Using a murine cutaneous model it

has been demonstrated that biofilms of *S. aureus* and *S. epidermidis* delay healing (Schierle et al, 2009). It has been suggested that biofilms of *P. aeruginosa* can impede wound healing by producing a rhamnolipid, which inhibits neutrophil function, preventing their ingress into the biofilm and inhibiting subsequent removal of the bacteria (Bjarnsholt et al, 2008).

VJ: Microorganisms will colonise the surface of the wound and release extracellular polymers (e.g. polysaccharides) that form a shield against host defense mechanisms. This shield can prevent the action of macrophages, impede immune mechanisms and interfere with the biological molecules involved in wound healing. This will cause the wound to become static and non-healing. The biofilm can also shield against the action of antibiotics or topical antimicrobial agents.

In your opinion, what is the best way to remove a biofilm?

RC: There is no ideal way to remove a biofilm from a wound and they are difficult to control because of their reduced susceptibility to antimicrobial agents. A strategy

called 'biofilm-based wound care' has been suggested, where sharp debridement and antimicrobial therapy are used together (Wolcott and Rhoads, 2008). There is some plausibility to this strategy — debridement aims to reduce the extent of a biofilm and the effectiveness of antimicrobial interventions are increased when microbes seek to regenerate the biofilm. This is because active growth makes them more susceptible to systemic antibiotics and topical agents. Innovative ways to prevent biofilm formation by interrupting quorum sensing are being investigated and clinical studies will be needed.

VJ: Biofilms can be disrupted by debridement or enzymes, followed by application of a topical antimicrobial agent to prevent reformation.

References

- Bjarnsholt T, Kirketerp-Møller K, et al (2008) Why chronic wounds fail to heal: a new hypothesis. *Wound Repair Regen* 16(1): 2–10
- Gristina AG, Price JL, Hobgood CD, Webb LX, Costerton JW (1985) Bacterial colonisation of percutaneous sutures. *Surgery* 98(1): 12–19
- Fazli M, Bjarnsholt T, Kirketerp-Møller K, et al (2009) Non-random distribution of *Pseudomonas aeruginosa* and *Staphylococcus aureus* in chronic wounds. *J Clin Microbiol* 47(12): 4084–89
- James GA, Swogger E, Wolcott R (2008) Biofilms in chronic wounds. *Wound Repair Regen* 16 (1): 37–44
- Kirketerp-Møller K, Jensen PØ, Fazli M, et al (2008) Distribution, organization and ecology of bacteria in chronic wounds. *J Clin Microbiol* 46(8): 2717–22
- Lovegrove JM (2004) Dental plaque revisited: bacteria associated with periodontal disease. *JNZ Soc Periodontol* 87: 7–21
- Marsh PD, Bradshaw DJ (1995) Dental plaque as a biofilm. *J Ind Microbiol* 15(3): 169–75
- Marsh PD, Moter A, Devine DA (2011) Dental plaque biofilms: communities, conflict and control. *Periodontol* 55(1): 16–35
- Mertz PM, Patti JM, Marcin JJ, Marshall DA (1987) Model for studying bacterial adherence to skin wounds. *J Clin Microbiol* 25(9): 1601–04
- Serralta VW, Harrison-Balestra C, Cazzaniga AL, Davis SC, Mertz PM (2009) Lifestyles of bacteria in wounds. Presence of biofilms? *Wounds* 13: 29–34
- Schierle CF, De la Garza M, Mustoe TA, Galiano RD (2009) Staphylococcal biofilms impair wound healing by delaying reepithelialization in a murine cutaneous wound model. *Wounds Rep Regen* 17(3): 354–59
- Wolcott RD, Rhoads DD (2008) A study of biofilm-based management in subjects with critical limb ischaemia. *J Wound Care* 17(4): 145–55



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