Biofilms cause chronic infections

A very impressive group of scientists and clinicians representing the European Society of Clinical Microbiology and Infectious Diseases (ESMID) recently concluded that:

'Biofilms cause chronic infections...'[1]

More important to wound care providers is this group, including individuals from across Europe and the US, used chronic wounds as one of their two examples of a biofilm infection, as evidence that biofilms are ubiquitous in human chronic non-healing wounds keeps increasing.[2,3] That a chronic wound should be viewed as a chronic infection is well documented science for the ESMID group[4,5] and as Terry Swanson reports in her survey in this issue (page 426), most wound care providers are in agreement with chronic wounds as biofilm infections at many levels. For one survey question 'The presence of biofilm can be a barrier to wound healing' over 90% of respondents indicated that they believed the statement is true/think this is true. Furthermore, it is also true that microbes, even growing as biofilm, which cause harm to the patient such as interfering with wound healing are causing a genuine infection. But biofilm infections are quite different to the single microbe infections (acute infections) with which we are familiar.

Briefly, biofilm is a different strategy microbes use to survive, a different mode of growth and a different pattern of gene expression. In a biofilm, up to one third of the microbe's genome (~800 previously dormant genes) are expressed.[6] The newly expressed genes and gene products are responsible for tolerance to antibiotics,[7] causing persistent inflammation[8] and effective evasion of host adaptive and innate immunity.[9] Yet no single gene or gene product can be used to identify microbes growing as a biofilm. A biofilm is usually composed of several different microbial species (polymicrobial)[10] that cooperate (synergy)[11] to cause infection. The variable microbial species which makes up the biofilm once attached (any tissue/organ in the body can be infected with biofilm) the biofilm diffuses and injects through multiple secretion systems agents.[12] These secretions are most often small peptides which render host cells senescent (alive but not functioning correctly), white blood cell dysfunction and persistent inflammation. A biofilm infection is parasitic in strategy and tries to maintain a sustainable niche for a long duration requiring the microbial community to subvert host healing pathways. The behaviours of a chronic wound, which we see every day in the clinic, such as exudate, slough and impaired healing can easily be explained by the natural infection methods of biofilm.

The wound microbiota producing infection, mainly biofilm infection, is an important cause of chronic wounds and this barrier must be effectively managed.

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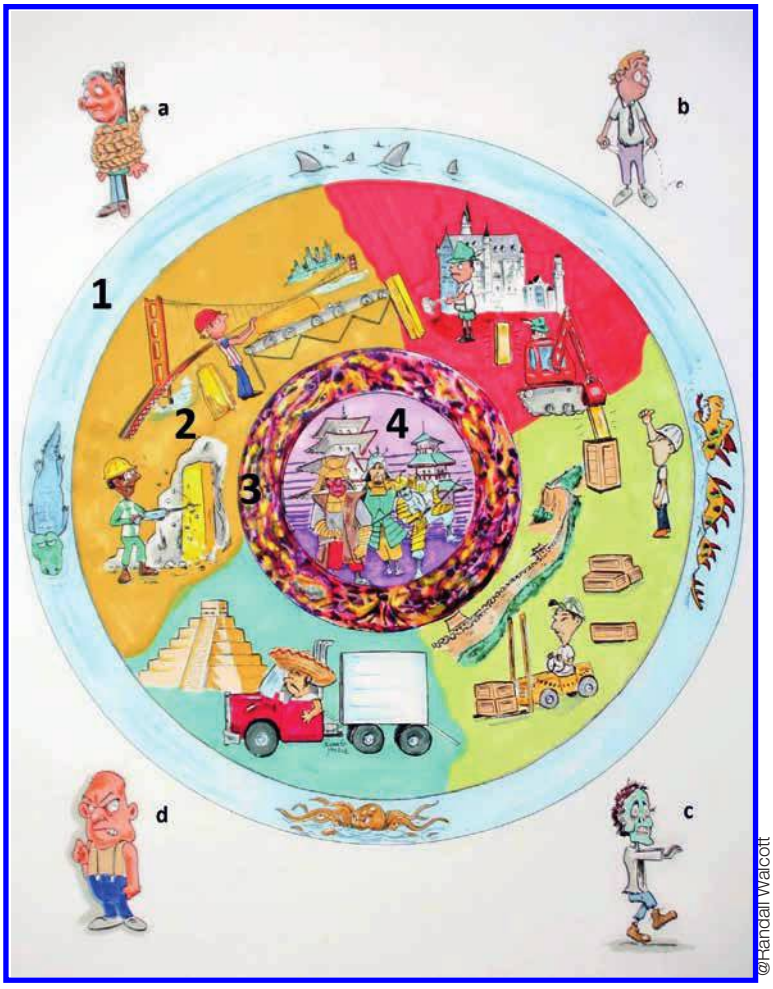
Wound care providers currently use many different antimicrobial strategies (antibiotics, biocides, physical disruption) by different methods (equipment, topical, systemic) with only small improvement in outcomes. The biggest barrier to effective biofilm management is that the main diagnostic tool, clinical culture is ill suited for diagnosing (microbial identification) biofilm infections.[13] Culture methods are over 150 years old and are not designed for biofilm infection because biofilms are usually multiple species in a mode where the microbial cells are alive but will not replicate in culture conditions (viable but will not divide so can not be cultured). Because of the severe inadequacies of culture methods wound care providers have evolved into a trial and error paradigm for antimicrobial management. Environmental and many other branches of microbiology long ago turned to nonculture methods to identify biofilm bacteria. Methods using the polymerase chain reaction (PCR), sequencing and mass spectrometry are now superior to culture and allow specific diagnosis and accurate monitoring of antimicrobial treatments. Dr Rennie reports in this issue (page 452) a possible new diagnostic hand held tool which identifies molecules excitable by a specific wavelength of light that are unique to bacterial species. This may allow for point-of-service diagnosis of if/where bacteria or in the wound.

New and multiple microbial diagnostics will pave the way for more effective wound care treatments. The current method of trying a single product then sequentially progressing through individual products in an ad hoc manner must give way to a solid scientific diagnose followed by a treatment standard. Modern medicine demands wound care to make this change. A once and done treatment regimen of antibiotics, an antimicrobial dressing or physical agents will rarely be enough to treat a biofilm infection.

The nature of biofilm infection requires more than a single treatment strategy. Emerging wound care methodologies use multiple treatments targeting the wound microbiota which are used synergistically to collapse the wound biofilm. Treatments such as sharp debridement, agents that degrade the biofilm matrix, agents that block biofilm communications (quorum sensing inhibitors), methods to rapidly remove exudate thus robbing the biofilm of nutrients, bactericidal agents and antibiotics are carefully chosen based on diagnostic identification of the wound microbiota and so as not to negatively interact. This treatment strategy for wounds is similar to many cancer treatments which start with maximum therapy using multiple agents and then deescalating (stopping individual agents/treatments) as

treatment goals are met. Therefore new treatments are needed to address the different complexities of a protected polymicrobial infection. The *Journal of Wound Care* (*JWC*) and its reviewers have carefully vetted many of these new treatments, several which are in this issue. Dr Wiegand and colleagues shows the efficacy of cold atmospheric pressure plasma technology against common wound microbes (page 462). Also Halstead et al. demonstrate the *in vitro* the ability of an engineered honey to degrade preformed biofilms from 16 well known wound pathogens (page 442). Their work documents exposure to honey decreased biofilm mass, disrupted biofilm structure and reduced biofilm seeding.

The complexity of diagnosing multiple different bacterial species (most of which we have never encountered before in the clinic), of learning multiple different treating agents specific to the 'weird bugs' (physical, bactericides, antibiotics) and then weave this all into a coherent treatment regimen seems daunting. But we should not be overwhelmed because we have much experience with biofilm in our daily lives. Every day we brush our teeth to manage a low grade biofilm infection called dental plaque with toothpaste composed of multiple antibiofilm agents. And there are many more examples. Plus all that we have learned from other chronic infections such as dental, cystic fibrosis, chronic sinusitis, and others will translate to wound microbiota. It is important that we accept this challenge to better understand biofilm infection, the diagnosis of biofilm microbes and especially the technologies and treatments that are emerging so that we can improve wound healing outcomes. **JWC**



A schematic diagram used to explain the actions of biofilm

1 Moat: This 'moat' represents a coating (matrix) that the biofilm community secretes and builds around the entire community. This coating is made up of sugars, proteins, lipids, DNA and many other polymers.

2 The cooperative community: Multiple different species of bacteria live within the biofilm. These different species cooperate to make the whole community more successful. This cooperation can be through shared nutrients, passive protection and functional equivalent pathogroups. It is synergy between microbial species that adds much to the resistance of biofilm to antibiotics and antiseptics.

3 The anoxic core: Biofilm has been demonstrated to have an anoxic core, this is an area where oxygen is depleted by the bacteria and metabolic waste products accumulate. To survive here individual bacterial cells must go dormant (no cellular activity) and resistant to traditional antibiotics which poison cellular activities, making biofilm up to 1000 times more resistant to antibiotics.

4 Quorum sensing: Quorum sensing molecules are the warlords of the biofilm. These molecules direct the different cells of the many bacterial species to express different genes. Bacterial cells that are near the surface (moat) must be metabolically active to sense the environment and to secrete diffusable molecules. However, bacterial cells deeper in the biofilm near the anoxic core must be dormant. Quorum sensing molecules direct these different patterns of gene expression throughout the biofilm.

Biofilm infection: In a wound the functions listed above in a biofilm are directed towards releasing small molecules into the wound bed. These small molecules cause the activity of the four men illustrated.

The Tied Up Man: This man demonstrates wound biofilm's ability to disrupt the cytoskeleton within the wound bed cells. This prevents our cells from shedding or migrating. This is wound biofilm's method for stabilising the wound bed and securing its attachment to the wound.

Poor Man: The wound biofilm's ability to block many intracellular activities within our host cells. This makes our cells unable to manufacture the products necessary for healing such as new blood vessels or laying down new proteins.

Zombie: This man represents one the wound biofilm bacterial cells ability to either inject or diffuse small molecules into our cells blocking the ability of our wound bed cells to die. The wound biofilm immortalises the cells that are on the surface of the wound bed, preventing shedding and to stabilise the attachment of the biofilm on the wound itself. Biofilm can much more quickly reestablish itself on senescent immortalised cells than it can on an active healthy host tissue.

The Angry Man: Wound biofilm has a problem if it stabilises the wound bed. What will it use as a nutrient source to propagate and spread itself out into the environment? It has overcome this by inflaming the wound bed, through diffusable molecules, hence the capillaries become leaky, plasma can then exit the capillaries into the wound bed and the biofilm can extract iron and other nutrient sources from the plasma.

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