Bacterial biofilms in wounds

Abstract

Bacterial biofilms are extremely common in the natural environment, and biofilms are known to cause chronic inflammation that contributes to the molecular pathologies of many diseases including periodontal disease, surgical device infections, urinary catheter infections, cystic fibrosis, chronic otitis media, and contact lens associated corneal infections. Compared to planktonic (free-floating) bacteria, coaggregated surface attached (sessile) microcolonies of bacteria in biofilms have enhanced resistance to killing by endogenous antibodies and phagocytic cells, as well as by exogenous antibiotics and antisepsics. Recent investigations indicate that many chronic skin wounds (~60%) contain bacterial biofilms, which suggests that biofilms play important roles in maintaining a chronic inflammation state that ultimately leads to the failure of skin wounds to heal. The terms critical colonisation or localised infection, which were created to describe wounds that fail to heal even with low numbers of planktonic bacteria (≤ 10⁵ colony forming units, CFUs), may actually be describing wounds that have biofilms. Removal of biofilms by debridement and prevention of reformation of biofilms by topical antisepsics and antimicrobial dressings may be the optimal treatment to move chronic wounds out of a chronic inflammatory phase and into a healing repair phase.

Effect of planktonic bacteria on wound healing

It has been hypothesised that the pathogenesis of chronic wounds has four main causative factors, which can occur alone or in combinations: tissue hypoxia, repetitive and injurious ischaemia-reperfusion, an impaired stress response due to age, and elevated bacterial levels. Normal, intact skin can contain substantial numbers of cultivable bacteria (≤ 10⁵ CFUs) without causing clinical problems. In open wounds, however, the presence of > 10⁵ CFUs of cultivable bacteria per gram of tissue (biopsies) is generally accepted as a diagnosis of clinical infection (or the strong probability of developing an infection). Clinical signs of infection may include inflammation (reddenning and/or heat particularly at the wound edge), increasing pain and tenderness (particularly around the wound site), excessive or purulent drainage, foul odour, and wound breakdown. Of the classical signs of infection, increasing pain and wound breakdown have been shown to be positive indicators of infection in chronic wounds. Clinical studies generally indicate that successful healing of acute wounds depends on maintaining a planktonic bacterial load ≤ 10⁵ CFUs per gram of tissue. Maintaining this reduced level of planktonic bacteria in a wound bed usually requires frequent, aggressive washing, debridement, and antimicrobial treatments (i.e. oral and/or topical antibiotics, antisepsics: Ag+, I₂, HOCl-, phenol, CHG, PHMB, etc). However, the ≤ 10⁵ CFU guideline does not always correlate with healing, since many chronic wounds have < 10⁵ CFU per gram of tissue, as measured by standard clinical microbiology techniques, do not show the most obvious clinical signs of infection, and yet still do not heal. Furthermore, clinical studies indicate that the extent of polymicrobial diversity of cultivable bacteria (four or more different species) or the presence of some highly virulent species of bacteria, such as beta haemolytic Streptococci in a chronic wound are key factors in determining whether a wound will heal.

Critical colonisation in chronic wounds

As illustrated in Figure 1, these clinical observations have led to the concept of critical colonisation, or local infection, which is intended to describe the state between normal colonisation, that does not cause delayed healing, and frank infection that clearly impairs healing. The diagnosis of delayed healing due to critical colonisation of a wound is frequently made retrospectively, after debridement and antimicrobial treatments are found to effectively promote healing of the wound. Thus, critical colonisation or localised infection are actually only functional terms that are used to describe the clinical condition that exists in wounds that fail to heal due to the presence of bacteria yet in the absence of clear signs of infection. But the key question remains, what is the actual microbial status of the wound bed in critically colonised wounds? Our hypothesis is that the clinical condition described as critical colonisation, or localised infection, is actually caused in large part by the presence of bacterial biofilms in the wound bed.

Structure and properties of microbial biofilms

As illustrated in Figure 1, free-floating planktonic bacteria initially attach reversibly to solid surfaces or adhere to each other (coaggregate) at surface interfaces (i.e. air-water) through weak interactions via bacterial components (e.g. fimbriae) and adhesion molecules. Under conditions of complete nutritional abundance and no chemical or physical environmental stress, bacteria will continue to proliferate at their maximum rate as planktonic bacteria. However, if bacteria sense any environmental or nutritional stress,
their natural response is to shift into a biofilm phenotype and begin to secrete various extracellular matrix components.11,13 The extracellular matrix components act as a kind of glue to more firmly attach the bacteria (sessile) to the surface, leading to the formation of structured microcolonies.14,15 Bacteria produce quorum sensing molecules, which accumulate in the environment as they multiply. Bacteria use the quorum sensing molecules to communicate with each other, causing substantial changes in the patterns of gene expression, that help the bacteria adapt to environmental changes and to develop biofilms.16−18 For example, as early as 15 minutes after initial attachment of P aeruginosa to a surface interface, the gene algC is activated, which is required for the synthesis of an exopolysaccharide (polygalinic acid) − the major component of Pseudomonas extracellular matrix.19 The polymicrobial (multiple bacterial species) nature of mature wound biofilms provides shared defensive advantages, such as secretion of proteins that provide antibiotic resistance to neighbouring bacterial species in the biofilm, or secretion of exopolysaccharides that shield the biofilm community from phagocytosis.20,21 In particular niches of the biofilm, local limitations of nutrients and oxygen may provide growth advantages to facultative anaerobes, or even strict anaerobes.22 In other niches of the biofilm, some bacteria essentially stop growing and become quiescent. The reduced metabolic activity of the quiescent bacteria is one mechanism that is thought to contribute to the greatly increased tolerance to certain antibiotics.23−26 Finally, bacteria in biofilms are often stimulated by environmental cues to dissociate from the exopolymeric matrix as free-floating, motile, planktonic cells or to break off as small fragments of the biofilm that can spread (seed) to new surfaces.11,25,30

**Increased resistance of biofilm bacteria to antimicrobials**

Most antibiotic treatments for patient care are based on the minimum inhibitory concentration (MIC) required to inhibit growth of planktonic bacteria that are cultured on nutrient rich agar plates. However, one property that typically distinguishes planktonic bacteria from biofilm bacteria is the greatly increased resistance of bacteria in biofilms to agents that normally kill planktonic bacteria very effectively. Examples reported in the literature include increased resistance to natural antibodies, phagocytic inflammatory cells (neutrophils and macrophages), antibiotic drugs, antiseptics, and disinfectants. The increased resistance is due to multiple factors as described above: limited diffusion of the microbial agents through a dense, highly negatively charged exopolymeric matrix that consists of polysaccharides, DNA and proteins; induced expression of specific biofilm genes required to produce special efflux pumps in bacterial cell membranes to pump out antimicrobial agents or secretion of molecules and enzymes that bind or inactivate antimicrobial agents; metabolically quiescent subpopulation of bacteria; creation of unique niches in the biofilm where oxygen is limiting or absent.20,22,23,26−27,31−33

The increased resistance of bacteria in biofilms has led to the more recent studies to determine the minimal biofilm eliminating concentration (MBEC) of antibiotics.25,34 Thus, research has shown that the MBEC of many antibiotics actually exceeds the maximum prescription levels available for the antibiotics.23,32,33 Therefore, standard oral doses of those antibiotics, which effectively kill planktonic bacteria, have little or no antimicrobial effect on biofilm bacteria *in vivo*.

**Measurement of bacteria in wounds**

At some point in the clinical management of many wounds, the decision will be made to assess the level, species, or antibiotic sensitivities of bacteria in the wound. As described in the recent International Consensus Document on Wound Infection in Clinical Practice,10 common sampling techniques for wounds include swabbing, needle aspiration and wound biopsy. Wound swabbing is most widely used, but may be misled by detecting surface colonising bacteria rather than more deeply sited bacteria. In addition, swab sampling is inappropriate for obtaining anaerobic bacterial species. Among techniques for swabbing wounds, the most useful method may be the Levine technique, in which a swab is rotated over a 1 cm² area of the wound with sufficient pressure to express fluid from with the wound tissue.26 Regardless of the sampling technique, clinical microbiology laboratories use standardised methods that generally select for growth of planktonic cultivable bacteria. Typical clinical laboratory results will not completely assess bacteria in biofilms because a significant percentage require special cultivation techniques or they are impossible to cultivate with current methods.11,37,38 Thus, clinically-practical means of detecting biofilms are lacking.

If standard clinical microbiology laboratory methods do not measure bacteria in biofilms, the question remains, how can clinicians assess if biofilms are present in a wound? Can bacterial biofilms be seen on a wound? The answer to this question is yes and no. Bacterial biofilm microcolonies are microscopic structures that cannot be seen by eye. However, if biofilms are allowed to grow to enormous size then they can be seen by eye. Examples of visible biofilms are the dental plaque that forms on our teeth, the coating that develops on urinary catheters, the deposits on extended wear contact lenses.
or the thin layer of viscous, slimy material on the surface of chronic wounds. Some bacterial biofilm can be visually detected because they produce pigments only when growing in a biofilm state (e.g. green pyocyanin of *Pseudomonas*). However, guidelines and standards of good wound care call for the removal (debridement) of such material from wounds. Furthermore, histological assessment of biopsies from chronic wounds frequently identified biofilm structures well below the surface of wounds. Thus, in many cases it seems that clinicians observe the consequences of biofilms in wounds (chronic inflammation, abundant exudate, fibrinous slough, failure to heal despite oral antibiotics) rather than observing the actual biofilms.

### Chronic wound management to promote healing

Currently, the most effective method of combating biofilms in both acute and chronic wounds is to remove the biofilms by debridement and use appropriate antimicrobial treatments to prevent the reformation of the biofilms. This concept is part of the process of Wound Bed Preparation, which is a comprehensive, systematic approach that integrates four basic aspects of wound care that promote healing by correcting barriers to healing. These four areas are covered by the acronym TIME, which identifies the need to remove necrotic tissue and existing biofilm. There are no bacteria that are shed onto the wound.

Clinical practice is to remove bacterial biofilms by an appropriate debridement techniques in removing biofilms, but it is probable that gentle wound irrigation will not effectively remove biofilms that are located beneath the wound surface. Furthermore, applying topical antibiotics without adequate debridement of biofilms is not sufficient to eliminate biofilms. This point is illustrated by an experiment where topical treatment of pig skin wounds with two common antimicrobial agents, mupirocin cream or triple antibiotic ointment (bacitracin zinc, polymyxm B sulfate, neomycin), effectively eliminated planktonic *S aureus* growing in the wounds after two days exposure, but the antibiotics had little effect on *S aureus* biofilms attached on the wounds even after four days of exposure. Thus, the key point for clinical practice is to remove bacterial biofilms by an appropriate method of debridement and prevent the reformation of biofilms by using therapies, when necessary, that effectively kill planktonic bacteria that are shed onto the wound.

### Summary

The contributions of bacterial biofilms in causing pathologies in multiple diseases are well established. The roles of bacterial biofilms in establishing and maintaining chronic wounds are just now being investigated, but initial results suggest that biofilms also play key roles in chronic wounds. From the clinician’s perspective, bacterial-based wound care does not require a totally new approach to optimal wound care. Rather, clinicians only need to continue to employ the principles of Wound Bed Preparation, but with an added emphasis on effective and frequent debridement of wounds and the control of infection and inflammation, which together remove established biofilms and prevent their reformation by planktonic bacteria.

### Declaration

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### References


