Assessing the bioburden in poorly healing wounds

Diagnosing when a wound is critically colonised can be difficult because these wounds do not display classical signs of infection and do not have clearly elevated levels of bioburden. Recent research suggests that the critically colonised state of a wound may be more precisely described as a colonisation by polymicrobial biofilm communities. Biofilm-based wound care emphasises the debridement of biofilms combined with the use of bacterial barrier dressings that prevent their rapid reformation.

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This article was first published in *Wounds International* (2011; 2(2):17–19).

Assessment of the bioburden of wounds has traditionally relied upon relatively simple microbiology laboratory techniques that typically use swabs or biopsies to provide information on the planktonic forms of major bacterial or fungal species. This technique has unquestionably generated important data that has been used for decades to help select therapeutic regimens for patients and their wounds.

The presence of $>10^5$ colony-forming units (CFUs) per gram of tissue (as demonstrated by biopsy) is generally accepted as a guideline for diagnosing a clinically infected wound. However, additional factors influence whether a wound will actually heal, including[1–4]:

- Presence of more than four different bacterial species (highly polymicrobial status of the wound)
- Presence of highly virulent species of bacteria (eg beta haemolytic streptococci)
- Ability of the patient to mount an effective inflammatory response (immune suppressed or immune compromised patients).

In the past, when these indicators were present, a clinical diagnosis of wound infection could be made with relatively high confidence. However, a major problem arose when these classical indicators of wound infection were not found, yet the wound did not heal after standard clinical care. Furthermore, these wounds often began healing when debrided and treated with systemic antibiotics or topical antimicrobial treatments and dressings[5].

This led to the concept of 'critical colonisation' or 'localised infection' to describe a delayed or non-healing response in a wound, where although the wound did not demonstrate classical signs of infection, the healing was definitely impaired by bacterial bioburden[2,6].

However, it is not clear what aspect of this relatively low total bioburden is 'critical' to impairing healing.

More thorough evaluation of standard clinical microbiology assays led to the realisation that their ability to culture all the bacterial and fungal species that are actually present in a chronic wound is inherently limited because they selectively grow planktonic bacteria that can multiply on simple agar media plates at 37°C. In other words, standard clinical microbiology assays are only able to culture a relatively small subset of the planktonic bacterial and fungal species present in a wound.

Thus, it seemed reasonable to assume that a more complete picture of the different bacterial (aerobic and anaerobic) and fungal species present in a particular wound would improve the ability of clinicians to assess the microbial bioburden in individual wounds and to indicate which therapeutic strategies would be optimal for each wound.

Current research

Recent laboratory research using electron microscopic examination of biopsies

References

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identified bacterial biofilm structures in a high percentage of chronic wounds (approximately 60%), but a very low percentage in healing acute wounds (approximately 5%)[7].

This finding takes on added clinical significance because of the well-documented high tolerance of mature polymicrobial biofilm communities to antibiotics, antiseptics and disinfectants[8]. Indeed, some studies suggest that it is not the total bacterial burden in wounds that impairs healing, but rather the presence of biofilm and perhaps even the presence of specific species in the biofilm[9,10].

In other words, the 'critically colonised' state of some wounds may actually be more precisely described as colonisation by polymicrobial biofilms that are not detected by standard clinical microbiology assays. These biofilms, like planktonic bacteria, stimulate chronic inflammation, leading to elevated levels of proteases and reactive oxygen species that can degrade those proteins in the wound that are essential for healing[11].

Research developments

To address the problem of incomplete identification of bacterial and fungal species by traditional laboratory culturing techniques, researchers and clinicians have attempted to identify those species present in wound samples based on their unique DNA sequences.

The only reason this approach is feasible is because of the tremendous advances that have been made in rapid polymerase chain reaction (PCR) assays, combined with the genomic nucleotide sequencing of thousands of bacteria and fungi in the last decade.

Using open access deoxyribonucleic acid (DNA) databases and advanced pyrosequencing techniques, a pivotal paper published by Dowd et al[12] in 2008 reported that the bacterial and fungal complexity of chronic wound samples was much greater than previously thought.

Researchers found that, on average, approximately 60% of the bacterial species present in chronic pressure ulcers and approximately 30% of those found in diabetic ulcers were strict anaerobic bacteria, and in fact many bacterial species were present that had never been reported before in cultures of chronic wounds.

This data suggests that many of the bacteria present in chronic wound biofilms could never be successfully cultured in a standard clinical microbiology laboratory due to obligate cooperation with other bacteria, creating unique environmental conditions in a polymicrobial community (the biofilm itself).

These results led to the development of a commercially available service that uses multiplexed PCR to identify approximately 30 of the most prevalent bacterial and fungal species in wound biopsies within 24 hours of receipt of a wound sample (PathoGenius Laboratories, Lubbock Texas – http://www.pathogenius.com/index-5.html).

A second major research development in the last year was the work by Wolcott el al, which demonstrated how mature biofilms are rapidly re-established in chronic wounds following surgical debridement[13]. This research indicates that sharp debridement opens a time-dependent therapeutic window to prevent the re-establishment of mature biofilms that are highly tolerant to host inflammatory response or to antimicrobial treatments.

Influential research

As well as the Wolcott et al paper mentioned above[13], a paper by James et al was the first to identify the presence of bacterial biofilm in chronic skin wounds and show that biofilms occur in a high percentage (approximately 60%) of chronic wounds, in contrast to a very low percentage (approximately 5%) of healing acute wounds[7]. This research compelled clinicians and researchers to begin thinking about bacterial bioburden in chronic wounds from a new perspective.

Future focus

Laboratory and clinical studies are underway to develop methods for topographically localising biofilms on the surface of a chronic wound bed, which would prevent the accidental debridement of healthy granulation tissue and help assess the effectiveness of various treatments in debriding and/or destroying biofilm bacteria in wounds. These studies should provide clinicians with better methods for reducing biofilms and preventing their reformation in wounds.

In addition, laboratory and clinical studies are underway to perfect rapid, point-of-care detectors for matrix metalloproteinases (MMPs) and nitric oxide. These two elements are the major downstream products of biofilm-induced inflammation and impair healing by degrading the proteins that are essential for wound healing.

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Expert Commentary

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Although our interest as clinicians in biofilms is increasing, it is generally regarded as a topic of interest rather than a determining element in the treatment of infectious disease. It has been estimated that 65% of all human infectious diseases, including wound infection, are due to biofilms[1] and that more than 17 million biofilm infections arise every year in the USA[2].

Phillips et al[3] put forward that chronic wounds are colonised by polymicrobial biofilm communities despite the absence of classic signs of infection. These communities are microscopic structures that cannot usually be detected by the eye unless they are capable of producing pigments in a similar manner to the green pyocyanin of *Pseudomonas*[4]. Research has confirmed that traditional laboratory techniques that depend on wound swabs and biopsies are designed to detect only planktonic bacteria capable of being cultivated and not the bacteria present in biofilms[5]. Bacterial cells in biofilms tend to alter their gene expression to adapt to their environment and thus cannot be grown on agar plates[6]. Recent research on using polymerase chain reaction (PCR) assays to identify bacterial DNA and usage of 16S ribosomal RNA sequencing has shown its accuracy in detecting bacterial cells in biofilms and determining their sensitivity to antimicrobial agents, compared with traditional culturing techniques[7].

Currently, wound care guidelines and standards call for the debridement of the wound bed to remove undetectable biofilm communities from the wound[8]. This revolutionary shift from using respected culture techniques to adopting molecular techniques should bring about change in infection control strategies and wound care guidelines, and direct healthcare providers in managing pathogenic biofim bacteria using the appropriate treatment. Despite this advancement within the microbiology laboratory field, a point-of-care detector for biofilms based on observation and experience is necessary to ensure early detection and the initiation of treatment; this is vital for improved outcomes for patients.

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