Wound infection

***Wounds International's* clinical innovations section presents recent developments in wound care. This issue, we focus on innovations in wound infection.**

Wound infection and diagnostics in practice: what is emerging?

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Wound infection unquestionably impairs healing, but many chronic wounds do not have high levels of planktonic bacteria as measured by standard clinical microbiology laboratory culturing methods. Recent research (James et al, 2008; Phillips et al, 2010) suggests this "critical colonisation" state is due to the presence of polymicrobial biofilm communities that are highly tolerant of hosting antibodies, inflammatory cells, antibiotics, and many antiseptics.

Polymerase chain reaction (PCR)-based identification of multiple bacterial species from wound biopsies appears to be a promising technology that overcomes most of the limitations of traditional bacterial-culturing methods. Methods of utilising improved profiling bacterial and fungal species present in wounds enables customised formulations of antibiotics and other agents that target a specific spectrum of organisms. Topical treatment with personalised antimicrobial formulations appears to substantially improve the healing of complex wounds (Dowd et al, 2011).

A rapid, point-of-care (POC) detector that assesses matrix metalloproteinase (MMP) activity identifies a subpopulation of chronic wounds (approximately 28%) that have a high probability (approximately 90%) of not healing. This could lead to targeted treatments with protease modulating therapies that may increase the chance of healing in those wounds (Serena et al, 2011). POC diagnostic platforms currently under development may be able to simultaneously measure levels of multiple biomarker proteins of impaired healing, as well as numerous bacterial and fungal species in wound fluid samples. The future of wound diagnostics, therefore, looks very promising indeed.

As summarised in the recent consensus document on wound infection in clinical practice (Harding et al, 2008), this area continues to be a hugely challenging one for clinicians and places a considerable burden on health services. The early recognition of a wound, along with prompt, appropriate, and effective intervention, is integral in reducing adverse economic and health consequences, especially in the context of growing levels of antibiotic resistance (Harding et al, 2008). Unfortunately, current standard clinical microbiology tests – which are based on the 100-year-old technique of growth of bacteria on nutrient agar plates – only provide a partial profile of the planktonic bacterial and fungal species present in wounds; essentially, those microorganisms adapted to grow rapidly under specific conditions in an incubator (Dowd et al, 2008). This leads, in most situations, to the identification of only a select few of the many planktonic bacteria present in a wound.

Clearly, there is a need for better diagnostic tests to identify and measure levels of bacteria and fungi in wounds. However, such a test (diagnostic) should meet several parameters. It should be cost-effective, at least in the range of the current standard clinical microbiology tests. Results should be generated in a few hours and provide information that can be used to guide clinical decision-making. In other words, just having more complete information about the bacteria and fungi species present in wound biopsies, curettes or swabs has minimal effect, unless there is a way to use that information to guide specific treatments for that patient, which is the aim underpinning the concept of personalised medicine.

Fortunately, molecular biology technologies have been developed that can replace the standard microbiology culturing technique. It quickly became evident in the 1990s that sequencing the 16S regions of bacteria and 18S region of fungi led to levels of sensitivity and specificity in identifying each microorganism present in a sample. Originally, the sequencing was accomplished using the pyrosequencing technique (Roche) because of its ability to produce long sequences of the 16S region, however, this technology was expensive. Important developments in bioinformatics and PCR techniques allowed for the accurate diagnosis and quantification of either a panel of key microbes (approximately 30) or identification of all the bacteria in a sample (Wolcott and Dowd, 2008).

Currently, it remains difficult to determine which of the bacterial or fungal constituents are implicated in the nonhealing of an individual wound. As PCR-based diagnostics become more established and larger databases are generated, it is highly likely that key patterns of bacteria will emerge that correlate with nonhealing. Also, the use of customised formulations of topical antibiotics and other agents will identify key treatable targets. Data from the co-author's (RDW) laboratory show that utilising personalised antibiotic

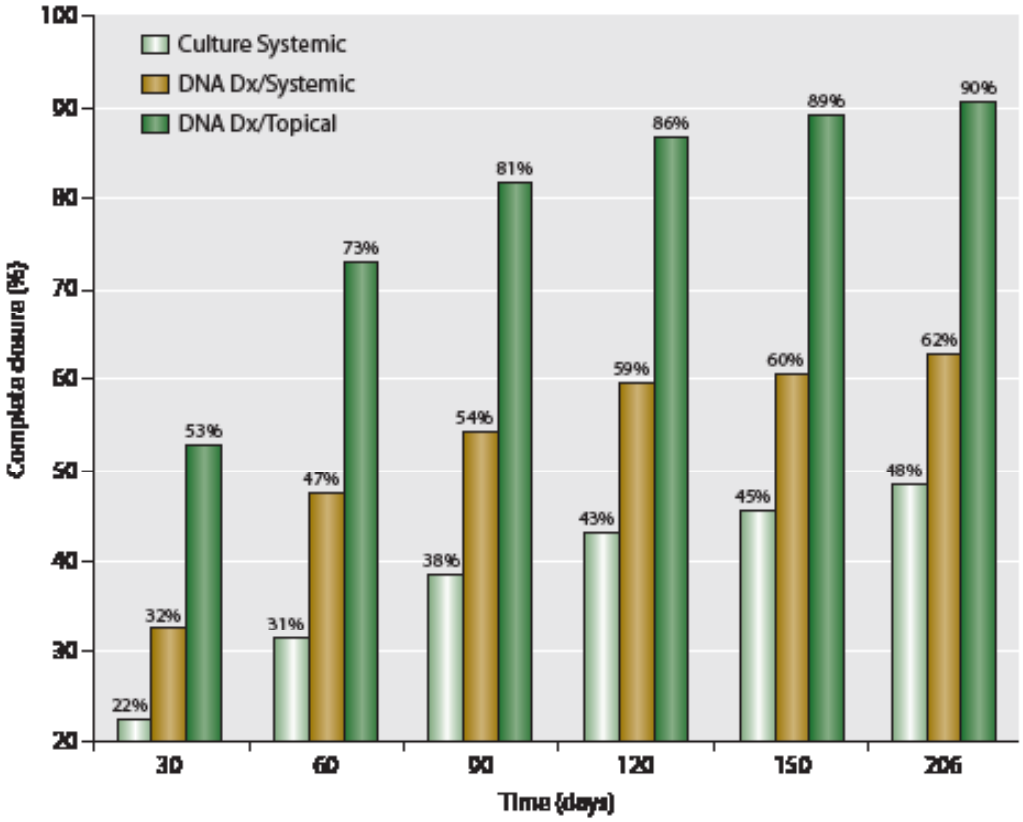

Figure 1. Improved healing of chronic wounds by personalised topical therapies guided by molecular diagnostics. A total of 1378 patients were treated by one of three protocols in Dowd et al (2011): Culture systemic, who received standard of care using systemic antibiotics on the basis of empiric and traditional culture-based methodologies; DNA Dx-Systemic group, who were prescribed an improved selection of systemic antibiotics based on the results of molecular diagnostics; DNA Dx-Topical group, who received personalised topical therapeutics (including antibiotics) based on the results of molecular diagnostics. Implementation of personalised topical therapeutics guided by molecular diagnosis resulted in statistically and clinically significant improvements in outcome.

gels prepared by a compounding pharmacy – containing multiple antibiotics targeting the 20–30 bacteria identified in a wound – collapsed the various populations to a level where two to three broad spectrum antibiotics could adequately manage the species present. Most importantly, healing rates of chronic wounds increased from 48% to 62%, following the implementation of molecular diagnostics and customised, targeted antibiotic regimens (Wolcott et al, 2010a).

In addition to the large number of different species of planktonic bacteria in chronic wounds, a high percentage of chronic wounds contain tightly attached polymicrobial biofilm communities that are not effectively cultured by the standard clinical microbiology assay techniques (James et al, 2008). Biofilms are known to cause chronic inflammation in several diseases, including periodontitis, osteomyelitis, cystic fibrosis, chronic otitis media, sinusitis, and Crohn's disease. This is due, in large part, to the fact that many of the polysaccharides, bacterial DNA, and proteins comprising the biofilm matrix stimulate both the innate immune system (toll-like receptors) and the adaptive immune system (antibodies). This has led some to hypothesise (Phillips et al, 2010) that biofilms are a major contributor to the prolonged inflammation that characterises most chronic wounds and leads to the clinical condition described as "critical colonisation" or "localised infection" in the spectrum of wound bioburden levels.

There is no specific diagnostic test for biofilms in chronic wounds at present, but there is clearly a need for a rapid, POC detector for biofilm communities in wounds. In the absence of such tests for biofilms in chronic wounds, wound care clinicians have had to rely on information from laboratories about the effects of antibiotics and antiseptics on biofilms, and from the outcomes of clinical treatment of biofilms in other diseases.

Bacteria in polymicrobial biofilms are extremely tolerant to the patient's own antibodies and phagocytic inflammatory cells, as well as oral antibiotics or topical antiseptics (Phillips et al, 2010). This is due to several factors: including reduced penetration of antibodies or antibiotics into the biofilm matrix; the reaction of antiseptic molecules with components of the biofilm matrix (the reaction-diffusion problem) (Stewart et al, 2001); and to the presence of quiescent persister bacteria that are not metabolically active in mature biofilm communities (Xu et al, 2000).

Given that essentially all antibiotics kill bacteria by interfering with some bacterial enzyme reaction, quiescent bacteria are not destroyed by the presence of antibiotics that are only able to kill bacteria when they are rapidly proliferating (metabolising). These challenges led to the concept of biofilm-based wound care (Wolcott and Rhoads, 2008).

The foundational principle of management of wound biofilm is debridement. Debridement contributes many positive aspects to the suppression of wound biofilms. First, there is physical disruption of the biofilm. It has been demonstrated that this forces the biofilm to reconstitute itself, which opens a time-sensitive window where the biofilm is more vulnerable to biocides and antibiotics. Thus, physically disrupting biofilm gives a 2- to 3-day period during which antimicrobials are more effective (Wolcott et al, 2010b). Second, combinations of agents in topical gels can attack multiple aspects of biofilms, such as inhibitors of quorum molecules that promote biofilm phenotypes in planktonic bacteria, and alcohol sugars, such as xylitol, that impair synthesis of the biofilm polysaccharides. Varying the composition of topical gels based on the bacterial species identified by DNA technologies should reduce the reformation of persistent biofilms.

An important molecular link between planktonic and biofilm bacteria that stimulate chronic inflammation is the elevated protease activities found in most chronic wound fluid and in dehisced acute wounds (Yager and Nwomeh, 1999; Ladwig et al, 2002; Utz et al, 2010; Gibson et al, 2010). This has led to the development of rapid, POC detectors for MMP activities in samples of wound fluids. Currently approved for use in most European counties, the WOUNDCHECK™ (Systagenix) diagnostic device detects the level of MMP activity in wound fluid samples. Current clinical results indicate that approximately 28% of nonhealing wounds have elevated protease activity levels and approximately 90% of those wounds will not heal without appropriate intervention, such as the use of a dressing that inhibits MMPs (Serena et al, 2011).

Other rapid, POC detector technologies are being developed. One of the most promising approaches appears to be in using modifications of surface plasmon resonance (SPR) technology to measure binding between two molecules (Lahav et al, 2004). SPR has several advantages as a diagnostic platform. It is a "label-free" detector system, which means that detection of a target molecule (biomarker) does not require a second labelled molecule (which is always needed in other detector systems, such as a lateral flow strip or an enzyme-linked immunosorbent assay). Also, multiple biomarker proteins can, in theory, be simultaneously measured in a sample using an SPR "chip" that contains multiple separate fields, each conjugated with an antibody to a different biomarker protein.

SPR signals can be generated in less than 10 minutes and detected using a simple illumination source and a spectrometer. Laboratory detection of bacterial species by SPR has been reported (Mazumdar et al, 2007; Baccar et al, 2010). Although much more research and development is required to produce a usable rapid, POC SPR detector for wound biomarkers and bacteria. Advances in SPR surface nanostructures and other components are currently occurring (Chung et al, 2010). The field of wound care may be entering a new phase of diagnostics for wound infection and biomarkers. ■

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