Micro-management: curbing chronic wound infection

Cathryn Withycombe[1], Kevin J. Purdy[2] and Sarah E. Maddocks[1*]

[1] Department of Biomedical Sciences, Cardiff School of Health Sciences, Cardiff Metropolitan University, CF5 2YB, United Kingdom

[2] School of Life Sciences, University of Warwick, Coventry, CV4 7AL, United Kingdom

*For correspondence: Sarah E. Maddocks, Department of Biomedical Science, Cardiff School of Health Sciences, Cardiff Metropolitan University, Western Avenue, Llandaff, Cardiff CF5 2YB, United Kingdom. Tel: 44-2920-20-5607. Email: smaddocks@cardiffmet.ac.uk.

Abstract

Chronic wounds, including pressure ulcers, foot ulcers and venous leg ulcers have a detrimental impact on the health and well-being of an estimated 2% of people in the UK. Chronic wounds are normally colonised by bacteria and in some instances bacterial load increases sufficiently for infection to ensue. Once a chronic wound becomes infected it is difficult to resolve and a combination of continuous inflammation and bacterial proliferation makes these wounds difficult to manage. A state of prolonged inflammation can occur as a result of impaired homeostatic pathways which are exacerbated by bacterial growth. Chronic, infected wounds can persist for many months or even years, sometimes requiring surgical intervention in the form of regular debridement or amputation when other strategies such as antimicrobial treatments fail. The complex relationships between both oral microbiota and the host have been extensively characterised, including the shift from health to disease, and has allowed for the development of numerous control strategies. This knowledge combined with contemporary studies of chronic infected wounds can be used to develop an understanding of the relationship between the host and microorganism in the chronic wound environment. Such information has the potential to inform wound management including strategies to control infection and promote wound healing.

Introduction

In developed countries, it has been estimated that 1 to 2% of the population will experience a chronic wound during their lifetime (Gottrup, 2004). Chronic wounds are defined as those that fail to heal in a predictable or timely manner (less than three months), being instead confined to one or more phases of wound healing (Werdin et al., 2009). Arrested healing perpetuates an ideal environment for microbial growth and all chronic wounds are colonised with bacteria; a state of infection occurs only when the microbial load exceeds critical colonisation ($\sim$1x10^6 CFU per gram tissue) (Kingsley, 2001).

Traditional culture and contemporary molecular diagnostics have identified diverse microbiota from chronic infected wounds, many of which are of endogenous origin (Singer and Clark, 1999). Most commonly isolated are *Staphylococcus aureus* and *Pseudomonas aeruginosa* (>90% and 80%, respectively) followed by *Enterococcus faecalis,* coagulase negative staphylococci and *Proteus spp.* Other common wound isolates include members of the genera *Enterobacter, Streptococcus, Citrobacter, Morganella* and *Corynebacteria* (Dowd et al., 2008, Frank et al., 2009, Wolcott et al., 2009, Wolcott et al., 2015*).*

Treatment of chronic infected wounds remains challenging with numerous strategies employed, including topical antimicrobial intervention, physical cleaning of the wound or surgical debridement (Kirshen et al., 2006, Jones et al., 2007, Game et al., 2012). Consequently, chronic wound management is difficult and infection recurs, often persisting for many years. Infection management from the host perspective is comparably complex as microorganisms have evolved with their host and adapted to immune defences. Through a combination of immune evasion, virulence and biofilm formation pathogens can persist within wounds and impair healing.

This review will consider how the microbial community and host environment contribute to sustained chronic wound infection and will evaluate how novel antimicrobial strategies might assist in management of chronic wound infection.

The changing host environment and onset of wound chronicity

Wound healing is a dynamic process that can be divided into haemostasis, inflammation, proliferation/epithelialisation and remodelling phases (Figure 1). Acute wounds manifest in a variety of ways including those with considerable tissue loss, incisional wounds and partial thickness wounds. In order to heal, each of these wound types follows the same series of complex, converging events, requiring the coordination of several cell types. Platelets provide the first response to wounding and neutrophils subsequently remove debris and bacteria. Mast cells signal the start of the inflammatory stage, which involves macrophages, fibroblasts, smooth muscle cells and lymphocytes. Post-inflammation, fibroblast, epithelial and endothelial cells proliferate, producing factors that encourage angiogenesis culminating with cross-linking of collagen and scar maturation (Diegelmann and Evans, 2004).

Situations can arise where healing progresses either in a deficient or excessive manner. An acute wound can become stalled and "stuck" in one of the four phases of the wound healing cycle, leading to what is referred to as a chronic or non-healing wound (Diegelmann and Evans, 2004). In a wound with excessive healing, contracture occurs (Inoue et al., 1998, Nedelec et al., 2000) which imposes stress on the wounded area and confines movement of the underlying tissues. There are various types of chronic wounds classified by a number of factors including aetiology, morphological characteristics and level of microbial contamination (Figure 2).

The chemistry of the wound bed also contributes to the propensity for chronic wound development. If the inflammatory phase continues for an extended period of time excessive levels of inflammatory cytokines such as IL1-β, IL-8 and TNF-α, are achieved and cells in the wound bed become senescent. Additionally, receptors on the surface of cells such as keratinocytes and fibroblasts are reduced in number preventing signalling by essential growth factors. These include epidermal growth factor (EGF), transforming growth factor-α (TGF-α), fibroblast growth factor 2 (FGF2) and vascular endothelial growth factor (VEGF) (which are essential for re-epithelialisation and vascularisation of new tissues), and their cognate receptors EGFR (for both EGF and TGF-α), FGFR and KDR, respectively (Jiang et al., 2014, DiPersio et al., 2016). Compounding this are excessive levels of proteases produced in response to abnormally high levels of TGF-β; these destroy the aforementioned growth factors and their associated receptors, thus preventing normal repair and angiogenesis. Fundamentally, the breakdown of normal wound healing can occur at any number of the stages outlined above which, if not appropriately 'managed' by the host, leave the wound open to contamination from skin microbiota and environmental microorganisms, including pathogens.

Microbial influence on the shift to wound chronicity

The wound repair pathways, described above, can be disrupted by microorganisms. Even without clinical signs of infection, bacteria interfere with the healing process by disrupting critical signalling systems which result in excessive production of pro-inflammatory cytokines such as IL1-β, IL-8 and TNF-α (Seth et al., 2012a, Seth et al., 2012b). Bacterial-derived components such as lipopolysaccharide, lipoteichoic acid, toxins and other secreted or surface bound effectors prompt this response using a Toll-like receptor dependent

mechanism (Zhao et al., 2013, Ward et al., 2015, Miller, 2008) which have been documented for a number of wound-associated, biofilm organisms including *P. aeruginosa, Klebsiella pneumoniae* and *S. aureus.* These pro-inflammatory signals initiate wound repair, cell migration, angiogenesis and inflammation; this is a normal response to infection, but if unabated maintains widespread tissue damage.

Recent investigations have described a scenario in which bacterial biofilm disrupts typical host immune responses within infected wounds, in a manner that is distinct to that of planktonic organisms (Thurlow et al., 2011, Nguyen et al., 2013, Ward et al., 2015, Gogoi-Tiwari et al., 2015, Secor et al., 2011, Sadowska et al., 2013). Predominantly, these studies have utilised *S. aureus* as a model since it is the most commonly isolated wound pathogen, but a few studies have focussed on *P. aeruginosa,* recognised as the second most common wound pathogen (Seth et al., 2012a, Seth et al., 2012b). The use of mouse models to assess the function of TL2 and TL9 (ligands for both are present within the biofilm) indicate that Gram positive bacteria within a biofilm, such as *Staphylococcus aureus,* evade traditional bacterial recognition pathways therefore successfully avoiding the host immune response. Typical attenuation of the pro-inflammatory response includes diminished production of IL-1β, TNF-α, CXCL2 and CCL2 (Thurlow et al., 2011). Soluble bacterial factors mediate the effects described above, presumably secreted by the infecting bacteria. Macrophages are also known to have impaired function and migration when biofilm is present (Donlan, 2002, Wolcott et al., 2008). Significantly it has been found that macrophages associated with the biofilm surface are predominantly non-viable (Thurlow et al., 2011). Movement of viable macrophages into the biofilm structure is also diminished and cells that permeate the biofilm tend to have limited phagocytic capabilities as a consequence of large matrix components resulting in frustrated phagocytosis which eventually causes macrophage death

(Zhao et al., 2013, Cerca et al., 2006, Schommer et al., 2011). In this case the effect is not instigated by secreted bacterial factors, but is reliant on surface-bound structures that are recognised by macrophages.

Unique, biofilm-associated protein profiles are associated with attenuation of the host-immune response to infection (Tankersley et al., 2014, Prabhakara et al., 2011, Kirker et al., 2009). Elevated apoptosis of keratinocytes and mesenchymal stromal cells combined with a general reduction in viability and impaired migration is observed in response to biofilms of a wide genera of microorganisms including *S. aureus* and *P. aeruginosa*, which ultimately impedes early stage angiogenesis and consequent wound healing (Kirker et al., 2009, Secor et al., 2011, Ward et al., 2015, Zhao et al., 2010) It has been proposed that these effects are mediated by reduced cytokine expression resulting from suppressed JNK (c-Jun N-terminal kinases) and p38 phosphorylation (Ward et al., 2015, Secor et al., 2011). A similar scenario is apparent for oral biofilm and it is conjectured that cytokines vital for initiating healing are degraded following biofilm contact (Fletcher et al., 1998, Guggenheim et al., 2009).

In vivo infections models incorporating medical conditions such as diabetes (which is associated with a high incidence of chronic ulceration of the lower limb) have modified the paradigms described above by inclusion of differing host characteristics (Falanga, 2005, Baltzis et al., 2014). For example in a TallyHo mouse model of type 2 diabetes in which pro-inflammatory cytokine expression is reduced, the TLR pathway for recognition of pathogens is impaired and re-epithelialisation disrupted (Nguyen et al., 2013). This could in part explain why individuals with underlying conditions that cause immune-suppression are more predisposed to developing chronic infected wounds, which are likely exacerbated

further by biofilm-mediated immune-dampening. Despite these recent advances in the understanding of bacterial biofilm and its role in chronic wound infection, the relationship between inflammatory response and clinical infection remains difficult to define. Currently bacterial bioburden remains to be the main indicator of progression towards infection (Grice et al., 2010). In light of this it is imperative to understand the microbial community that comprises the bacterial bioburden.

Microbial communities in the chronic wound

The skin microbiome is comprised of four major phyla (Acinteobacteria, Bacteroidetes, Firmicutes and Proteobacteria) which also form a significant proportion of the oral and gastrointestinal microbiota (Grice and Segre, 2011). Within these environments interpersonal species variation differs significantly but temporal community stability endures relatively unchanged. Wounds facilitate a dramatic shift from a dry environment to one that is comparatively wet causing a substantial environmental modification which has a significant impact on the composition of the microbial community.

Microbiota of the skin is uniquely adapted to withstand low water availability, acidic pH, sloughing of the epidermis layer and enzymatic secretions such as lysozyme. The majority of skin microorganisms are classified as commensals or opportunistic pathogens normally inhabiting the host without detriment. Invariably, following damage to the skin microorganisms residing therein colonise the exposed tissues. Much like microorganisms of the oral cavity, skin- and wound-associated bacteria have an abundance of surface bound receptors that confer upon them the ability to attach to cell-associated proteins and components of the extracellular matrix (Romero-Steiner et al., 1990, Darmstadt et al., 1999, Mempel et al., 1998, Coates et al., 2014).

Following skin damage, non-differentiated keratinocytes and epithelial cells, and fibroblasts are exposed along with an abundance of ECM-associated proteins providing new surfaces and different ligands for bacterial attachment (Santoro and Gaudino, 2005). Aerobic and anaerobic members of the endogenous microbiota adhere better to differentiated cells indicating that a niche consisting of non-differentiated cells might be available for colonisation by exogenous microorganisms expressing appropriate surface adhesins (Romero-Steiner et al., 1990). Several bacterial surface adhesins have been identified that are critical for interaction with either keratinocytes or epithelial cells of the skin including the Eap protein of *S. aureus,* which is overexpressed *in situ* in wounds (Thompson et al., 2010, Palma et al., 1999, Hussain et al., 2002), streptococcal M protein (Darmstadt et al., 2000, Okada et al., 1995), various fibronectin and collagen binding proteins and the Bap protein of *Acinetobacter baumanii,* which is critical for biofilm formation (Brossard and Campagnari, 2012). Gram positive and Gram negative bacteria are covered in a plethora of surface proteins that function as adhesins. Within the context of the wound environment these adhesins mediate attachment to ubiquitous host proteins such as collagen, fibronectin, vitronectin, elastin and laminin found on the cell surface and within the extracellular matrix. Consequently, there is likely to be competition between colonising bacteria for attachment. Competition for attachment sites is likely to be less competitive where highly specific interactions have evolved, for example between the M protein of *Streptococcus pyogenes* and CD44 on human keratinocytes.

The application of traditional ecological theory to the human microbiome and infectious disease remains relatively new. However, it is generally accepted that niche characteristics at the site of infection confer selective pressures responsible in part, for shaping the microbial community. Numerous studies describe an archetypal model in which

microorganisms compete for resources, resulting in the emergence of so called social "cheats" (Morgan et al., 2012, Harrison and Buckling, 2009). Contrary to this is the notion that synergy is critical for biofilm community composition and microbial survival. This advocates an alternative hypothesis in which the dynamics of the developing microbial community can be better described in terms of proliferation and association rather than selection and competition. Significantly bacterial association is observed during the development of dental plaque and the resulting community co-operation is known to be integral for the onset of gingivitis and tooth decay (Hajishengallis and Lamont, 2012, Filoche et al., 2010, Sbordone and Bortolaia, 2003, Kolenbrander et al., 2006b). At present, equivalent evidence describing wound colonisation and the onset of infection, is insubstantial.

Despite this, several *in vivo* and *in vitro* studies have described the basic interactions between several pathogens within the wound biofilm. Molecular investigations have identified prominent members of the biofilm using a combination of 16S rRNA gene analysis, denaturing gradient gel electrophoresis, fluorescent *in situ* hybridisation and pyrosequencing (Frank et al., 2009, Wolcott et al., 2009, Dowd et al., 2008, James et al., 2008, Kirketerp-Møller et al., 2008). These studies have revealed typical chronic wound biofilms to be comprised of species of *Staphylococcus, Pseudomonas, Enterobacter, Stenotrophomonas, Corynebacterium* and *Clostridium*. Of these *Staphylococcus* is most prevalent, followed by *Corynebacterium, Clostridium* and *Pseudomonas* with other microorganisms present in lower numbers. The bacterial species that colonise wounds tend to be similar between patients and irrespective of the type of wound there is an observable reduction in microbial diversity over time. The latter phenomenon is evident in other types of chronic infection including the cystic fibrosis lung and oral cavity, suggesting a correlation

between chronicity and diminished microbial diversity (Coburn et al., 2015, Kirst et al., 2015). Reduced microbial diversity within a given environment can occur as a consequence of successive colonisation. Favourable environmental conditions ultimately lead to the emergence a small number of dominant species whose growth alters the local environment thus conferring a competitive growth advantage. This model has been used to describe the developing oral biofilm and as a fuller understanding of wound colonisation emerges, might become applicable to the development of wound biofilm and management of chronic wound infection (Teles et al., 2012).

Can oral microbial communities serve as a model for chronic wound infection?

The oral microbiome is one of the most extensively characterised human microbial communities. Despite considerable diversity of species, oral biofilm development follows a well-defined, temporal course (Kolenbrander et al., 2006a). The composition of healthy oral biofilm can change if left undisturbed, and this change can ultimately lead to gingivitis and/or periodontitis. Unlike the colonised wound, the initiation of periodontal disease is independent of a critical colonisation threshold. Instead dysbiosis underpins the shift from health to disease with the predominance of anaerobic organisms serving as indicators of disease (Hajishengallis and Lamont, 2012, Lamont and Hajishengallis, 2015, Berezow and Darveau, 2011). Typical microbial profiles associated with "normal skin microbiota" are sometimes observed within chronic wounds but once infection has established, these diminish to be replace by a few pathogenic bacteria; this is indicative of dysbiosis as observed in the oral cavity. Critically, the factors that initiate and continue to drive changes in microbial community composition and pave the way to chronic wound infection remain

to be clarified. As such, no temporal description of biofilm development in the chronic wound exists, to date, that would aid understanding of dysbiosis and the onset of disease.

Early plaque is characterised by the presence of cocci and short rods of relatively conserved phyla. Co-aggregation between pioneer and secondary colonisers that comprise these phyla is crucial and mixed-species colonies emerge within a very short space of time (Kolenbrander, 1988). Cocci and rods of the genera *Staphylococcus, Streptococcus, Propionibacterium, Corynebacterium* and *Acinetobacter* frequently colonise the skin and wound-bed, where they form microcolonies. Given their proximity to wounded or damaged skin, it is likely that these microorganisms serve as early or pioneer colonisers in wound biofilms. At present there is little information describing specific bacterial interaction in wound biofilms due to a lack of suitable models. However, understanding such processes is fundamental for the appropriate, targeted intervention strategies for prophylactic management of wound infection.

Environmental factors play a central role in microbial population dynamics, exerting various selective, evolutionary pressures. Biofilm within the oral cavity is dynamic, experiencing conditions of flow and regular disruption; consequently, rapid attachment and co-aggregation is paramount for successful colonisation. Flow is negligible within wounds and is estimated at between 0.41-0.52g/cm^2/24h (no infection) and 0.87-1.2g/cm^2/24h (infected wound) (Thomas et al., 2014) far lower than that of the oral cavity (0.48ml/min) (Fenoll-Palomares et al., 2004). The low to negligible flow rate within the wound suggests a less dynamic environment which might not select for rapid bacterial attachment and co-aggregation. For example, bacterial microcolonies have been observed within wounds within ten hours following contamination, whereas for dental plaque on freshly cleaned

tooth surfaces, this occurs within four to eight hours (Palmer et al., 2003). Despite this disparity co-aggregation to form microcolonies is an integral process for biofilm development for two reasons. Firstly it mediates early attachment and microcolony formation at the sub-stratum; secondly it provides a competitive growth advantage to pre-aggregated bacteria that subsequently attach to the developing biofilm (Alhede et al., 2011).

Co-operation is evident in oral biofilms and relies on the altruistic behaviour of member organisms such as the streptococci who undergo lysis to release DNA which is incorporated as a structural component of the growing biofilm as eDNA (Liao et al., 2014, Klein et al., 2015). As well as stabilising the biofilm, eDNA also has an important role in horizontal gene transfer. Whilst this seemingly altruistic behaviour is not unique to biofilms of the oral cavity, no reports have described this phenomenon within the wound biofilm. However, given the ubiquity of eDNA as a scaffold molecule within biofilms and its observation within single-species biofilms of *P. aeruginosa* and *S. aureus,* it is not unreasonable to hypothesise that eDNA forms an integral component of wound biofilms. A growing body of evidence indicates synergy of virulence between members of wound biofilms including *S. aureus* and *P. aeruginosa.* For example *S. aureus* is known to enhance the growth of host adapted strains of *P. aeruginosa* and promotes an S*. aureus* small colony variant phenotype (Mitchell et al., 2010, Mashburn et al., 2005b). Additionally *P. aeruginosa* is known to utilise *S. aureus* as a source of iron and can also promote the expression of *S. aureus* virulence factors such as Panton-Valentine leucocidin (Mashburn et al., 2005a, Pastar et al., 2013). Therefore, early co-operation in addition to eventual dysbiosis is a fundamental consideration for chronic wound management.

Despite much being understood about the bacterial interactions within oral biofilm communities, at present not enough is understood about chronic wound biofilm communities for these models to be applied as a means of describing the ecology of the chronic infected wound. However, this breadth of knowledge can be used to inform and build accurate models of chronic wound infection. A wider theoretical template for biofilm development in chronic wounds that takes into consideration the shift from healthy colonisation to disease, has the potential to be clinically useful. For example, understanding temporal biofilm development with regards to specific bacterial interactions could inform appropriate, timely intervention where clinical infection is apparent but laboratory diagnosis is not yet confirmed. In short it could allow the clinician to intervene to prevent secondary infection and make better decisions about topical wound treatments which are pertinent to chronic wound management. For a fuller review of the application of knowledge gleaned from the study of oral biofilms, to wound biofilms the reader is directed to Mancl *et al.,* 2013.

Managing chronic wound infection with antimicrobials

In the UK topical antibiotics are not usually utilised for the treatment of wound infection and systemic antibiotic treatments are not routinely administered due to problems associated with targeting of treatments to the site of infection. However, for severe recurrent infection with associated biofilm current guidelines support the extended use of high dose, orally administered antibiotics which still has limited success (Hoiby et al., 2015). Consequently, antiseptics are still a front-line solution applied in the form of creams or ointments, or impregnated into wound dressings (www.cochrane.org). Regardless of this many antimicrobial treatments remain ineffectual resulting in more drastic strategies such

as submersion in potassium permanganate, hydrogen peroxide or bleaches as a last resort prior to life-changing procedures such as amputation (Wounds International, 2008).

Topical treatments often do not resolve chronic wound infection because they diffuse poorly through the wound-bed and extracellular polysaccharide layer of the biofilm and therefore do not reach all infectious organisms. This consequently results in the establishment of concentration gradients which expose microorganisms deep within the biofilm to sub-lethal doses of antimicrobial treatment, imparting a selective evolutionary pressure that favours the emergence of resistant phenotypes (Percival et al., 2011). Therefore, following completion of antimicrobial treatment infection often recurs. To counter these problems numerous novel strategies have been developed to ensure appropriate delivery of efficacious treatments that do not promote antimicrobial resistance. Many of these are currently not available to medical practitioners and remain in the early stages of development.

Attractive alternatives to traditional antimicrobial treatments include quorum sensing inhibitors (Njoroge and Sperandio, 2009), anti-biofilm/anti-adhesive (Rabin et al., 2015) or anti-virulence compounds (Stubben et al., 2009), nano-formulated antimicrobials (Neethirajan et al., 2014) and combination therapies drawn from natural products including phenolic compounds and flavonoids (Borges et al., 2015). The appeal of many of these therapies is their ability to attenuate virulence, which does not impose traditional "survival of the fittest" pressures upon the microbial population. With regards to biofilm associated infection such as chronic wound infection, anti-biofilm/anti-adhesive strategies could provide an effective solution.

A growing number of varied compounds demonstrate anti-biofilm activity. Of particular note is an emerging family of cationic antimicrobial peptides with poor microbial killing but good anti-biofilm activity, being especially effective at penetrating and dispersing established or mature biofilms (Park et al., 2011). Specific amino acid motifs (FRIRVRV) associated with anti-biofilm property, have been identified. Although their precise function remains to be determined these peptides impair swimming, twitching and swarming motility in *P. aeruginosa* as well as supressing the expression of genes involved in biofilm formation (Xu et al., 2014). This knowledge provides a basis for the synthesis of targeted anti-biofilm peptides and given that several cationic anti-microbial peptides are currently licenced for medical use; they represent a tangible alternative treatment for biofilm infection in wounds.

Jamming microbial communication can also impair biofilm development and attenuate virulence. The majority of quorum sensing inhibitors that have so far been developed rely on *P. aeruginosa* a model system and are therefore more applicable to control of infection by Gram negative bacteria (Starkey et al., 2014). However early analysis of peptide quorum sensing molecules derived from Gram positive bacteria indicate that this strategy could be more broadly applied. Significantly it is possible to impair the autoinducer three system, *in vitro* (Rasko et al., 2008). The autoinducer three quorum sensing system is necessary for inter-species bacterial signalling and therefore might prove effective for impairing the development of polymicrobial communities. But more specifically, studies using *E. coli* have indicated the involvement of QseC (which is part of the autoinducer three system conserved in Gram negative bacteria), also responds to human catecholamine hormones which results in attenuated virulence (Rasko et al., 2008). Anti-biofilm and quorum-sensing inhibition are both strategies that rely on disruption of the microbial

community, impeding their interactions with the host to promote clearance of infection rather than mediating bacterial death.

In recent years there has been significant focus on natural products as a source of novel antimicrobial compounds. Plant derivatives are rich in anti-oxidants, flavonoids and polyphenols all of which are known to impair bacterial growth (Savoia, 2012). Despite numerous studies which have isolated and identified individual antimicrobial compounds from these sources, it is generally believed that the antimicrobial efficacy of natural products can be attributed to the combined activity of different components, similar to the hurdle technologies utilised for microbial control within the food industry. Whilst natural products offer an alternative route to identify new antimicrobials beyond synthetic chemistry, they ultimately impose the same selective pressures as traditional antimicrobials and equivalent challenges with regards to effective delivery. Within the multitude of emerging natural antimicrobial treatments, several with anti-biofilm or anti-virulence properties have been identified. These properties are often only evident at sub-lethal doses and appear secondary to bacterial lysis or impaired growth. Consequently, using such compounds at levels that impart non-lethal mechanisms of infection control could inevitably result in resistance in the long term.

Novel delivery systems have tried to overcome problems associated with antimicrobial delivery and nano-formulation is a promising strategy. Nano-formulated antimicrobials do not necessarily have a different mode of action but when incorporated into antimicrobial wound dressings or polymers, for example have a slower and therefore prolonged rate of release therefore ensuring a steady dose over an appropriate time frame to resolve infection (Zhang et al., 2010). The versatility of nano-formulated antimicrobial

means they can be incorporated into a large variety of different materials from wound dressings to polymers and dental materials (Hook et al., 2014, Wood et al., 2014, Barbour et al., 2013). However, the disadvantage is that antimicrobial nano-particles have so far utilised bactericidal compounds such as silver or chlorhexidine, which does not counter problems associated with antimicrobial resistance. Despite this promising research has revealed that peptides can be packaged into nano-structures which allow for topical delivery (Bi et al., 2011). With regards to chronic wound infection, this would allow peptides to be delivered to the site of infection, and could be adapted to utilise novel antimicrobial peptides, such as those with anti-biofilm activity. Furthermore, hydrogels developed specifically for chronic wound treatment, can be loaded with nanoparticles for efficacious delivery to the wound site whilst maintaining an environment that is conducive to wound healing (Villanueva et al., 2016, Das et al., 2015, Ng et al., 2014).

Despite an apparent abundance of newly explored and emerging antimicrobial treatments, managing chronic infected wounds still remains a challenge. A variety of treatments are relied upon for the management of chronic wound infection and where traditional antimicrobials fail combined strategies are utilised that include wound cleaning, surgical debridement and in the most severe cases, amputation. Given the extreme, sometimes life-changing nature of interventions such as these, the drive to identify and develop alternate efficacious chronic wound management strategies is vital.

Summary and conclusions

Two contrasting hypotheses describe the chronic wound scenario. The first purporting that biofilm within the wound impairs healing and the second that the host is the cause of delayed wound healing with the development of biofilm being a natural consequence of the

failure to re-epithelialize in a timely manner. If chronic infected wounds are to be managed appropriately and effectively these concepts must not be considered as mutually exclusive. Therefore, understanding host-pathogen interactions during chronic wound infection is essential to enable knowledge about the development and treatment of chronic infected wounds to be translated into palpable clinical interventions.

Conflict of interest: Drs Withycombe, Maddocks and Purdy have nothing to disclose

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