Critical colonisation of chronic wounds: microbial mechanisms

Microorganisms found on the skin are usually regarded as innocuous symbiotic organisms (commensals), pathogens or potential pathogens. In recent years, we have radically revised our understanding of the host-microorganism interaction together with the mechanisms of bacterial virulence. Studies have shown that chronic wounds are colonised by multiple bacterial species, many of which persist in the wound. The presence of bacteria such as *Pseudomonas aeruginosa* can induce wound enlargement and/or delayed healing. It is this situation of delayed healing that we equate with critical colonisation.

Richard White, Keith Cutting

KEY WORDS
Delayed healing
Chronic wounds
Critical colonisation
Infection

Infection in chronic wounds presents a major clinical challenge and is a cause of high morbidity. Much attention has been given to identifying and managing this problem since the publication of the authors' article on chronic wound infection criteria (Cutting and Harding, 1994). Those pathogenic micro-organisms which cause wound infection have first to overcome a wide range of specific and non-specific antimicrobial mechanisms, and, phagocytic cells (polymorphonuclear neutrophils or PMNs) which form a crucial part of the innate host response against bacterial infection (Kobayashi et al, 2003). Invading bacteria become opsonised by complement proteins or antibodies and subsequently phagocytosed and killed by PMNs. To illustrate how alteration in wound bioburden impacts pathology, the Wound Infection Continuum (WIC) has been devised and subsequently modified (Gray et al, 2005). One stage in this continuum, critical colonisation, is putatively described as a pivotal phase that occurs without inducing an overt host response. Critical colonisation is better explained from a microbiological than from a clinical perspective. The status of critically colonised wounds may change in one of several ways: 1) deteriorate to clinical infection, 2) remain in a critically colonised state, or 3) improve following appropriate intervention. Research to clearly define the term and clarify the role of bioburden in the chronic wound is needed to help clinicians recognise and implement appropriate treatment.

The term critical colonisation has attracted increasing attention over the past five years. It has been regarded by a number of authors as synonymous with local infection (Edwards and Harding, 2004). Some have dismissed the concept of critical colonisation as a myth, expressing the view that a wound is either infected or not, with no prodromal phase of infection (Gilchrist, 2003). In order to advance understanding of delayed healing in the absence of an obvious clinical cause, the basic concept of critical colonisation deserves consideration.

If alternative explanations for delayed healing can be identified, patient morbidity can be reduced. Delayed healing must be placed accurately into context to help avoid making or perpetuating inappropriate assumptions. This article reviews the emergence of the concept of critical colonisation from an historical perspective, discusses assumptions that have been made, and presents scientific evidence collated from the literature. This approach draws some parallels with Cutting and Harding (1994), where a review of the literature led to the collation of traditional and additional diagnostic features of wound infection and the development of an entirely new approach to identifying clinical wound infection. These criteria have been refined by Cutting and White (2005).

Development of the concept

The term critical colonisation was first coined in 1996 by Davis. Using case studies, Davis demonstrated how delayed healing in wounds could be reversed through appropriate use of topical antiseptics. She also defined the condition of the wound in relation to bacterial presence. Using a modified model for infection first published by Ayton (1985), Davis introduced the notion of critical colonisation within the infection spectrum (from 'sterile', 'colonised', 'critically colonised', 'infected') and defined it as 'multiplication of organisms without invasion but interfering with wound healing.' Davis also stated that 'the classic signs of

Richard White is Professor of Tissue Viability, Institute of Health, Social Care and Psychology, University of Worcester, Worcester, and Keith Cutting is Principal Lecturer, Buckinghamshire New University, Chalfont St Giles

infection must be reassessed to include the "critically colonised" wound,' (1996) offering the first association with local infection. In support of her treatise, Davis cited Danielson (1994) and Trengove et al (1996), espousing the notion that the presence of pathogens, with or without host reaction, could interfere with healing. Currently, the absence of a host response is viewed as a fundamental link to understanding the concept of critical colonisation. Kingsley (2001) renamed the model of wound infection the 'Wound Infection Continuum'. This model is most closely associated with chronic wounds, where sterility and contamination are not clinically relevant and colonisation may be regarded as the 'normal' state.

While the term critical colonisation may sound novel, the underpinning concept has been part of the wound healing lexicon under various guises. A review of the literature in relation to delayed healing reveals the use of a number of synonymous phrases, including silent infection, covert infection, occult infection, refractory wound, subclinical infection, indolent wound (Kingsley, 2003), stunned wound, sub-acute infection, and recalcitrant wound.

Critical colonisation in clinical context

Role in the Wound Infection Continuum

With heightened focus on wound microbiology and infection in recent years, the Wound Infection Continuum has been proposed as a model to account for an increasing microbial load (bioburden) and related pathology (Kingsley, 2001; 2003). Although the concept of critical colonisation is not universally accepted, clinicians and researchers generally agree that the term needs definitive characterisation in order to validate its consideration in infection management (Ovington, 2003; Cutting and White, 2005).

Wound infection development depends on complex microbial and host factors with the latter being the governing factor. While many will claim quantitative bacterial values as a criterion for infection, the reliance on numbers is unjustified in chronic wounds (Bowler, 2003). An in vivo study (Trengove et al, 1994) has shown that the number of bacterial species and the number of organisms are important factors in the development of infection. However, these findings have yet to be clinically validated. In a theoretical hypothesis, Heinzelmann et al (2002) submit that the host response, or immune status of the individual, is a key factor in the development of infection; the triggering of a host response has been used as a diagnosis of infection for 2000 years.

For those who accept it as a distinct entity, critical colonisation is a stage where wound healing is delayed by microbial factors without the overt signs and symptoms of infection (Cutting, 2003); it occurs despite optimum treatment (Kingsley, 2003). It would appear to be a contradiction that a microbially induced delay in healing could occur without eliciting a host response. How could such a situation arise without the host response playing a part? A number of authors have reported delayed ulcer healing influenced by microorganisms: Lookingbill et al (1978), Daltrey et al (1981), Halbert et al (1992), and Hansson et al (1995). In a retrospective review of patients with various inflammatory wounds such as *necrobiosis lipoidica*, Drosou et al (2003) provide an additional perspective, stating it is likely that subclinical damage to tissue as a result of bacterial contamination exists and cites Hermanns et al (1999) in support of this premise.

Clinically, host response to wound infection is recognised by the classic signs and symptoms of inflammation, i.e. redness, swelling, warmth, and pain. Spreading erythema around the wound is usually indicative of infection such as erysipelas or cellulitis (Hansson et al, 1995). However, not all erythematous reactions are immunologically generated. Recent findings from a series of clinical cases have shown that Morganella species (notably *M. morganii*) commonly found in wounds express histamine in physiologically significant amounts (Cooper et al, 2004); therefore, in some wounds, periwound erythema could be attributable to *M. morganii* colonisation. This Gram-negative bacillus inhabits the gastro-intestinal tract and is a part of the normal faecal flora. It has been reported in chronic leg ulceration (Aspiroz et al, 2004) and in Chiclero's ulcer in a microbiological study involving 26 patients (Aspiroz et al, 2004), but is not routinely considered in bacterial samples acquired from wounds. Hansson

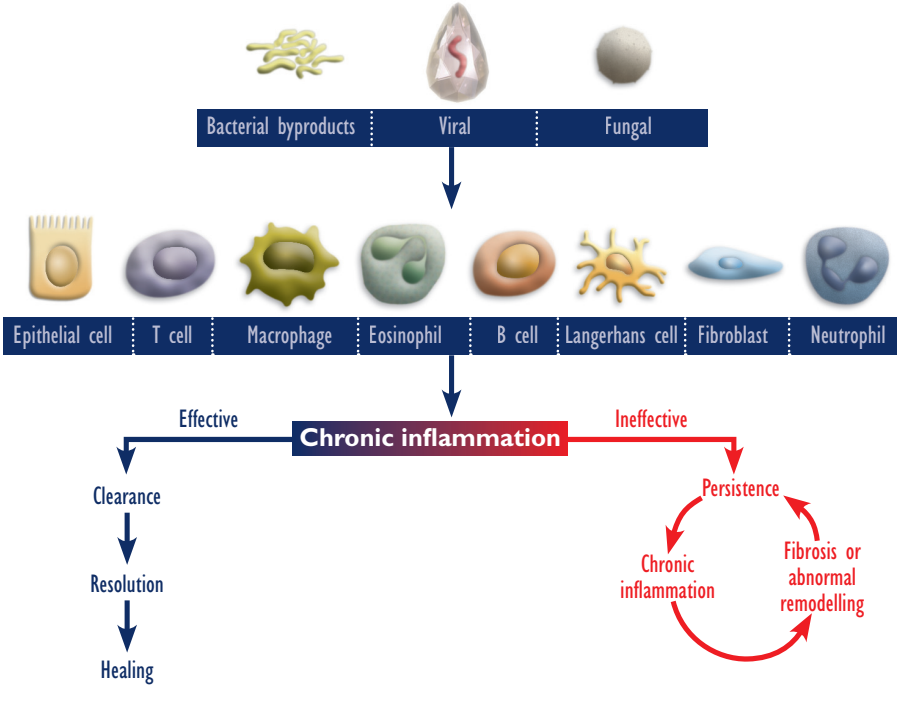

Figure 1. The influence of infectious agents on chronic wound inflammation and delayed healing.

Table 1.
Critical colonisation: factors involved in delayed healing

- Immuno-invasion
- Apoptosis
- Anti-inflammatory and anti-angiogenic
- Biofilm formation
- Cytotoxicity
- Chronic inflammation
- Trojan horse

et al (1995) found *M. morganii* (identified as *Proteus morganii*) in 23% of venous leg ulcers (n=58) studied. Conversely, Bowler and Davies (1999), in a review of data from a prospective clinical study where swabs from 44 infected leg ulcers were compared with 30 from non-infected ulcers, found this bacterium in 'infected' but not 'non-infected' leg ulcers. In this study, the diagnosis of ulcer infection was determined on the basis of clinical signs including erythema, cellulitis, oedema, increased pain, increased exudate, and warmth.

Microbial factors

In literature reviews of the cell biology of chronic wounds, delayed healing has been intimately linked with uncontrolled inflammation (Meneghin and Hogaboam, 2007; Eming et al, 2007; Menke et al, 2007) or immunopathology (Page et al, 2006). This is not visually evident in many chronic wounds because it is not always accompanied by the classical signs of inflammation. However, delayed healing is histologically evident (Abd-El-Aleem et al, 2005). The acolytes of critical colonisation believe delayed healing often can be attributed to microbial factors (Gray et al, 2005), and that frequently diagnosis is confirmed only retrospectively once antimicrobial measures have been taken and found to be effective.

How, then, can delayed healing be associated with microbial factors and not elicit an obvious host response? Three or more potential bacterial modes of action, described in the literature, can delay wound healing without any apparent inflammatory or immunological response: the expression of immuno-evasion (Allen et al, 2005), biofilm formation (Cooper and Okhiria, 2007; Davis et al, 2008; James et al, 2008), and suppression of cellular wound healing responses (Stephens et al, 2003). These modes have been identified following in vitro work and can occur when the wound is colonised by certain specific bacteria.

Pseudomonas aeruginosa

Pseudomonas aeruginosa is an important opportunistic human pathogen commonly found in chronic wounds (Bowler and Davis, 1999) and associated with chronic infection (Lau et al, 2004). It is known to form biofilms (Costerton, 2001) and secrete immuno-evasive factors (Usher et al, 2002) active against polymorphonucleocytes (PMNs). To this effect, activation of the type III secretion system, a recently identified virulence determinant of *P. aeruginosa*, has been reported from in vitro studies using clinical isolates (Dacheux et al, 2002). It has been postulated from in vitro studies that *P. aeruginosa* is likely to be of far greater significance to wound chronicity, tissue invasion, and infection than previously recognised (Serralta et al, 2001; King et al, 2003; Stephens et al, 2003; Allen et al, 2005; White, 2006). This is attributable in part to its capacity to form biofilms, and to produce exotoxins and enzymes such as elastase (Girard and Bloemberg, 2008). The quorum sensing strain PA01 produces elastase in response to homo-serine lactone, while increased pyocanin production (see below) may be regulated by other mechanisms (Le Berre et al, 2008).

As indicated in a summary of clinical and microbiology findings by Hamilton and Danielsen (1997), experienced wound clinicians have long noticed occasional green colouration in chronic wounds and have associated it with the presence of *P. aeruginosa* and delayed healing. Green wounds have been diagnosed as infected on the basis of colour alone (Benbow, 2007). This we now know to be inaccurate. The green pigment, pyocyanin, is a phenazine, a highly diffusible exotoxic metabolite described in an in vitro study by Denning et al (2003). The *P. aeruginosa*-derived phenazine pigment pyocyanin primes human neutrophils for release of superoxide and myeloperoxidase (Ras et al, 1992). In a review of published clinical and in vitro data by Lau et al (2004), pyocyanin has been shown to inhibit many cell functions and impair host defences through apoptosis. In vitro laboratory research on clinical samples (Zychlinsky and Sansonetti, 1997a) has shown that many pathogens, including *P. aeruginosa*, induce inappropriate or premature apoptosis (programmed cell death) of immune cells such as macrophages and neutrophils and that this can be pro-inflammatory (Zychlinsky and Sonsonetti, 1997b).

P. aeruginosa has evolved immuno-evasive strategies by which it affects host immunity (Buret and Cripps, 1993). In vitro studies have shown pyocyanin and other similar phenazines to have pro-apoptotic action on human neutrophils (Usher et al, 2002). This is postulated to be a clinically important mechanism of persistence of *P. aeruginosa* in human tissue (Usher et al, 2002). What mediates the change in the infective potential of this organism? *P. aeruginosa* has been shown to be a phenotypically unstable pathogen, particularly in chronic infection (Speert, 2002). The virulence of *P. aeruginosa* is controlled by an N-acyl homoserine lactone (AHL)-dependent quorum sensing system. The organism has been shown in vitro to have the capability to modulate its own quorum-sensing dependent pathogenic potential through an AHL-acylase enzyme (Sio et al, 2006). This may in part explain how under certain circumstances *P. aeruginosa* may be a delayer of wound healing and under other circumstances an infecting organism.

A parallel has been drawn between the chronicity of some wounds (venous leg ulcers, pressure ulcers, and diabetic foot ulcers) and cystic fibrosis (Jensen et al, 2007) insofar as *P. aeruginosa* is implicated in biofilm formation, polymorphonuclear neutrophil function, and a possible 'shielding' mechanism which protects the bacterium from phagocytosis (Hooi et al, 2004). The authors postulate that 'the presence of *P. aeruginosa* in

biofilms, and the lack of concomitant elimination by attended PMNs, are the main causes of inefficient eradication by antibiotic treatment and antimicrobial activity of the innate immune system, respectively' (Bjarnsholt et al, 2008). While this article relates to what the authors describe as 'infection in chronic wounds', it will apply equally to what has been described as 'critical colonisation'. Furthermore, in cystic fibrosis, *P. aeruginosa* is found with *Burkholderia cepacia*, possibly in symbiosis or synergy. This situation could be the case in chronic wounds where the synergy becomes one virulence determinant of many.

Other aerobes and anaerobes also have been recognised for down-regulating the immune response. In an in vitro microbiology study, Bowler et al (1999) summarised the role of succinate (a dicarboxylic acid) produced by aerobes and anaerobes; in vitro studies by Rotstein et al (1987; 1989) demonstrate how succinate may increase the risk of infection by impairing host cell function.

Escherichia coli is both a normal member of the intestinal flora, and, a prominent human pathogen causing a broad spectrum of diseases. It is routinely found in chronic wounds such as sacral pressure ulcers and venous leg ulcers (Brook and Frazier, 1998; Bowler and Davies, 1999). A mechanism whereby *E.coli* subverts the innate immune system has recently been described (Fexby et al, 2007). A surface protein on *E.coli* — antigen 43 (Ag43) — has been shown to promote bacterial binding to some human cells, biofilm formation, enhanced resistance towards antibacterial agents, and, the capacity to survive phagocytosis by PMNs in an opsonin-independent manner, in effect a 'Trojan horse' (Fexby et al, 2007). While this has yet to be demonstrated in wounds, it may provide part of the rationale for 'chronic' inflammation and for critical colonisation.

Staphylococcus aureus

Staphylococcus aureus is also an important human wound pathogen that interferes with host-cell functions. According to in vitro studies, impaired healing often is observed in *S. aureus*-infected wounds where the extracellular adherence protein (EAP) has been implicated (Athanasopoulos et al, 2006). Extracellular adherence protein has been shown in in-vitro studies to be a potent anti-inflammatory (Chavakis et al, 2002) and anti-angiogenic agent, preventing recruitment of inflammatory cells to the wound site as well as inhibiting neovascularisation (Haggar et al, 2004).

Odour-producing microorganisms

Wound malodour, a common characteristic of chronic wounds, has been linked to short-chain fatty acids (SCFAs) in in-vitro studies (Bowler et al, 1999). These volatile compounds are the metabolic by-products of anaerobic bacterial metabolism.

Malodour is associated with organisms known to generate SCFAs such as *Bacteroides spp* and anaerobic cocci (Haggar et al, 2004). In an in vitro study, Stephens et al (2003) demonstrated that Peptostreptococci-generated SCFA inhibited the growth of key cells responsible for wound healing, e.g. keratinocytes, fibroblasts, and endothelial cells. If translated to the in vivo situation, this could result in delayed healing from uncomplicated colonisation (i.e. no perceived clinical or cellular effects) without the bioburden necessarily reaching a theoretical infection threshold. Hansson et al's in-vivo study (1995) found Peptostreptococcus species (identified as *P. magnus*, *P. asaccharolyticus* and *P. prevotii*) in 30% of venous leg ulcers. This is a clinically significant level of species-specific colonisation and indicates the importance of anaerobic involvement in chronic wound bacteriology.

Short-chain fatty acids studies in vitro have been shown to play a part in impairing neutrophil chemotaxis and phagocytosis (Stephens et al, 2003). The low pH of all chronic wounds facilitates succinate activity and provides a milieu that down-regulates neutrophil function (Rotstein et al, 1987; 1989).

Differentiating critical colonisation as a distinct stage of infection

From the theses presented, it can be observed that a chronic wound colonised but not infected with one or more of certain bacteria (among them *Morganella spp*, *P. aeruginosa* and *Peptostreptococcus spp*) may exhibit erythema and delayed healing without a traditional or otherwise evident host response. Scenarios involving these organisms and possibly others yet to be identified have been used to postulate the concept of critical colonisation.

The critical nature of colonisation takes on a far greater significance when viewed in this light. A low level of colonisation may be all that is required to delay healing and is far removed from that required for local infection to be diagnosed in terms of the level of bioburden and the demonstration of signs of infection (host response). Simply renaming local infection as critical colonisation has no value (Bowler, 1998).

The fundamental message is that a number of possible mechanisms may allow microorganisms to contribute to delayed healing without overt signs of infection. This is not to be confused with the subtle signs of infection (Cutting and Harding, 1994). Critical colonisation is currently better explained from a microbiological perspective than from a clinical perspective. This should encourage clinicians to pay closer attention to delayed healing and its assessment. Currently, it has yet to be determined how frequently delayed healing can be attributed to a microbiological cause or to other factors. In chronic wounds, the fact that colonisation is the norm should precipitate the conclusion that delayed healing is more likely than not to be microbiological in origin.

When encountered clinically, delayed healing may be perceived as an idiosyncratic event that defies rational explanation. In the absence of firm evidence to explain delayed healing, e.g. malnutrition, smoking, comorbidities, less-than-optimal care, critical colonisation should be considered not as a confounding feature but as a clinical probability based on the rationale presented. To put this into context, many clinicians have seen an indolent wound improve following topical antimicrobial treatment,

retrospectively confirming the 'diagnosis' of critical colonisation.

Clearly, these are areas for research before mechanisms can be clearly defined. The concepts outlined in this article may offer a suitable starting point.

Conclusion

Wound microbiology, particularly in the so-called chronic wound, has justifiably achieved a high profile. While wound infection is a cause of morbidity and subsequent increased patient management costs, the state of delayed healing also presents cause for concern. Healing delays adversely affect patient quality of life and are used as justification for expensive modern wound treatments.

The term critical colonisation describes the situation of delayed healing with a microbial cause. It is likely that this state will vary between individuals and over time. It should be viewed microbiologically, not purely quantitatively but also qualitatively, where its manifestation is dependent on the species present and thereafter by the expression of virulence determinants by those species. The goal in such situations is to consider treatment such as topical antiseptics that control the bioburden so healing may proceed (White et al, 2005a, b). It is important to recognise that critical colonisation is a distinct, clinically important stage in the Wound Infection Continuum; not acknowledging that critical colonisation is a cause of delayed healing (even without a host response) impedes early diagnosis and appropriate treatment. Currently there exists clear demarcation between the stages of the Wound Infection Continuum model. This requires revision or perhaps the generation of an entirely new model if critical colonisation is to be accepted as a meaningful entity and placed correctly into clinical context. Additional studies need to ascertain the point at which a wound is critically colonised, as well as to identify appropriate treatment to avoid additional morbidity and care costs. **WUK**

This article has been modified from White R, Cutting K, Critical Colonization — The Concept under Scrutiny. Ostomy and Wound Management 52(11) November 2006: 50–56

References

Abd-El-Aleem SA, Morgan C, Ferguson MW, et al (2005) Spatial distribution of mast cells in chronic venous leg ulcers. *Eur J Histochem* **49(3)**: 265–72

Allen L, Dockrell DH, Pattery T, et al (2005) Pyocyanin production by *Pseudomonas aeruginosa* induces neutrophil apoptosis and impairs neutrophil-mediated host defenses in vivo. *J Immunol* **174(6)**: 3643–9

Athanasopoulos AN, Economopoulos M, Orlova V, et al (2006) The extracellular adherence protein (EAP) of *Staphylococcus aureus* inhibits wound healing by interfering with host defense and repair mechanisms. *Blood* **107(7)**: 2720–7

Aspiroz C, Navarro C, Aguilar E, Rodriguez-Andre M (2004) Bacteraemia in an obese patient with cellulitis and chronic ulceration in the lower extremity. *Enferm Infec Microbiol Clin* **22(6)**: 363–4

Ayton M (1985) Wounds that won't heal. *Nurs Times* **81(46 suppl)**: S16–S19

Benbow M (2007) Patient assessment and wounds. *J Community Nurs* **21(7)**: 18–22

Bjarnsholt T, Kirketerp-Møller K, Jensen PØ, et al (2008) Why chronic wounds will not heal: a novel hypothesis. *Wound Rep Regen* **16(1)**: 2–10

Bowler PG (1998) The aerobic and anaerobic microbiology of wounds: a review. *Wounds* **10(6)**: 170–8

Bowler PG, Davies BJ (1999) The microbiology of infected and non-infected leg ulcers. *Int J Dermatol* **38(8)**: 101–6

Bowler PG, Davies BJ, Jones SA (1999) Microbial involvement in chronic wound malodour. *J Wound Care* **8(5)**: 216–18

Bowler PG (2003) The 105 bacterial growth guideline: reassessing its clinical relevance in wound healing. *Ostomy/Wound Management* **49(1)**: 44–53

Brook I, Frazier EH (1998) Aerobic and anaerobic microbiology of chronic venous ulcers. *Int J Dermatol* **37(6)**: 426–8

Buret A, Cripps AW (1993) The immunoevasive activities of *Pseudomonas aeruginosa*. *Am Rev Respir Dis* **148(3)**: 793–805

Chavakis T, Hussain M, Kanse SM, et al (2002) *Staphylococcus aureus* extracellular adherence protein serves as an anti-inflammatory factor by inhibiting the recruitment of host leukocytes. *Nat Med* **8(7)**: 687–93

Costerton JW (2001) Cystic fibrosis pathogenesis and the role of biofilms in persistent infection. *Trends Microbiol* **9(2)**: 50–2

Cooper R, Okhiria O (2007) The issue of biofilms in wounds. In: White R, ed. *Advances in Wound Care, volume I*. HealthComm UK Ltd, trading as Wounds UK, Aberdeen: 189–204

Cooper RA, Morwood S, Burton N (2004) Histamine production by bacteria isolated from wounds. *J Infect* **49**: 39

Cutting KF (1998) The identification of infection in granulating wounds by registered nurses. *J Clin Nurs* **7(6)**: 539–46

Cutting KF (2003) Wound healing, bacteria and topical therapies. *EWMA J* **3(1)**: 17–19

Cutting KF, Harding KG (1994) Criteria for identifying wound infection. *J Wound Care* **3(4)**: 198–201

Cutting KF, White RJ (2005) Criteria for identifying wound infection — revisited. *Ostomy/Wound Management* **51(1)**: 28–34

Dacheux D, Epaulard O, de Groot A, et al (2002) Activation of Pseudomonas aeruginosa type III secretion system. *Infect Immun* **70(7)**: 3973–77

Daltrey DC, Rhodes B, Chattwood JG (1981) Investigation into the microbial flora of healing and non-healing decubitus ulcers. *J Clin Pathol* **34(7)**: 701–5

Danielson L (1994) The role of *Pseudomonas aeruginosa* in chronic wounds. Proceedings of the 4th European Wound Management Association Conference on Advances in Wound Management. Copenhagen, Denmark; September 6–9

Davis E (1996) Don't deny the chance to heal! Presented at the 2nd Joint Meeting of the Wound Healing Society and the European Tissue Repair Society, Boston, Mass; May

Davis SC, Ricotti C, Cazzaniga A et al (2008) Microscopic and physiologic evidence for biofilm-associated wound colonization in vivo. *Wound Rep Regen* 16(1): 23–9

Denning GM, Iyer SS, Reszka KJ, et al (2003) Phenazine-1-carboxylic acid, a secondary metabolite of *Pseudomonas aeruginosa*, alters expression of immunomodulatory proteins by human airway epithelial cells. *Am J Physiol Lung Cell Mol Physiol* **285(3)**: 584–92

Drosou A, Kirsner RS, Welsh E, Sullivan TP, Kerdel FA (2003) Use of infliximab, an anti-tumor necrosis alpha antibody, for inflammatory dermatoses. *J Cutan Med Surg* **7(5)**: 3823–86

Edwards R, Harding KG (2004) Bacteria and wound healing. *Curr Opin Infect Dis* **17(2)**: 91–6

Eming SA, Krieg T, Davidson JM (2007) Inflammation in wound repair: mollecular and cellular mechanisms. *J Invest Dermatol* **127(3)**: 514–25

Eron LJ, Lipsky B, Low D, et al (2003) Managing skin and soft tissue infections. *J Antimicrob Chemother* **52(1 suppl)**: S3–S17

Fexby S, Bjarnsholt T, Ostrup Jensen P, et al (2007) Biological Trojan horse: antigen 43 provides specific bacterial uptake and survival in human neutrophils. *Infect Immun* **75(1)**: 30–4

Gilchrist B (2003) Finding bacteria in wounds:

are you being misled? Presented at European Wound Management Association Conference Proceedings, Pisa, Italy, 22–24 May

Girard G, Bloemberg GV (2008) Central role of quorum sensing in regulating the production of pathogenicity factors in *Pseudomonas aeruginosa*. *Future Microbiol* **3(1)**: 97–106

Gray D, White RJ, Kingsley A, Cooper P (2005) Using the wound infection continuum to assess wound bioburden. *Wounds UK* **1(2 suppl)**: S15–S21

Haggar A, Ehrnfelt C, Holgersson J (2004) The extracellular adherence protein from *Staphylococcus aureus* inhibits neutrophil binding to endothelial cells. *Infect Immun* **72(10)**: 6164–67

Halbert AR, Stacey MC, Rohr JB, et al (1992) The effect of bacterial colonization on venous ulcer healing. *Aust J Dermatol* **33**: 75–80

Hamilton Jakobsen B, Danielsen L (1997) Venous leg ulcer. *Ugeskr Laeger* **159(19)**: 2836–40

Hansson C, Hoborn J, Moler A, Swanbeck G (1995) The microbial flora in venous leg ulcers without clinical signs of infection. *Acta Derm Venereol* **75(1)**: 24–30

Heinzelmann M, Scott M, Lam T (2002) Factors predisposing to bacterial invasion and infection. *Am J Surg* **183(2)**: 179–90

Hermanns JF, Paquet P, Arrese JE et al (1999) La cytotoxicité bénéfique des antiseptiques. *Rev Med Liege* **54(7)**: 600–5

Hooi DS, Bycroft BW, Chabra SR, et al (2004) Differential immune modulatory activity of *Pseudomonas aeruginosa* quorum-sensing signal molecules. *Infect Immun* **72(11)**: 6463–70

Isaac-Marquez AP, Lezama-Davila CM (2003) Detection of pathogenic bacteria in skin lesions of patients with Chiclero's ulcer. *Mem Inst Oswaldo Cruz* **98(8)**: 1093–5

James GA, Swogger E, Wolcott R et al (2008) Biofilms in chronic wounds. *Wound Rep Regen* 16(1): 37–44

Jensen PØ, Bjarnsholt T, Phipps R, et al (2007) Rapid necrotic killing of polymorphonuclear leukocytes is caused by quorum-sensing-controlled production of rhamnolipid by *Pseudomonas aeruginosa*. *Microbiol* **153(pt 5)**: 1329–38

King JR, Koerber AJ, Croft JM, et al (2003) Modelling host tissue degradation by extracellular bacterial pathogens. *Math Med Biol* **20(3)**: 227–60

Kingsley A (2001) A proactive approach to wound infection. *Nurs Standard* **15(30)**: 50–8

Kingsley AR (2003) The wound infection continuum and its application to clinical practice. *Ostomy/Wound Management* **49(7 suppl A)**: S1–S7

Kobayashi SD, Voyich JM, DeLeo FR (2003) Regulation of the neutrophil-mediated inflammatory response to infection. *Microbes Infect* **5**: 1337–44

Lau GW, Hassett DJ, Ran H, et al (2004) The role of pyocyanin in Pseudomonas aeruginosa infection. *Trends Mol Med* **10(12)**: 599–606

Le Berre R, Nguyen S, Nowark E et al (2008) Quorum-sensing activity and related virulence factors expression in clinically pathogenic isolates of *Pseudomonas aeruginosa*. *Clin Microbiol Infect* 7 Jan (Epub ahead of print)

Lookingbill D, Miller SH, Knowles RC (1978) Bacteriology of chronic leg ulcers. *Arch Dermatol* **114(12)**: 1765–8

Meneghin A, Hogaboam CM (2007) Infectious disease, the innate immune response, and fibrosis. *J Clin Invest* **117(3)**: 530–8

Menke NB, Ward KR, Witten TM, et al (2007) Impaired wound healing. *Clin Dermatol* **25(1)**: 19–25

Moore K (1999) The cell biology of chronic wounds: the role of inflammation. *J Wound Care* **8(7)**: 345–52

Ovington L (2003) Bacterial toxins and wound healing. *Ostomy/Wound Management* **49(7 suppl A)**: 8–12

Page KR, Scott AL, Manabe YC (2006) The expanding realm of heterologous immunity: friend or foe? *Cell Microbiol* **8(2)**: 185–96

Ras GJ, Anderson R, Taylor GW et al (1992) Clindamycin, erythomycin and roxithromycin inhibit the pro-inflammatory interactions of *Pseudomonas aeruginosa* pigments with human neutrophils in vitro. *Antimicrob Agents Chemother* **36(6)**: 1236–40

Rotstein OD, Nasmith PE, Grinstein S (1987) The bacteroides by-product succinic acid inhibits neutrophil respiratory burst by reducing intracellular pH. *Infect Immun* **55(4)**: 864–70

Rotstein OD, Vittorini T, Kao J, et al (1989) A soluble bacteroides by-product impairs phagocytic killing of *Escherichia coli* by neutrophils. *Infect Immun* **57(3)**: 745–53

Serralta VW, Harrison-Baletra C, Cazzaniga AL, et al (2001) Lifestyles of bacteria in wounds: presence of biofilms? *Wounds* **13(1)**: 29–34

Sio CF, Otten LG, Cool RH, et al (2006) Quorum quenching by an N-acyl-homoserine lactone acylase from *Pseudomonas aeruginosa* PA01. *Infect Immun* **74(3)**: 1673–82

Speert DP (2002) Molecular epidemiology of *Pseudomonas aeruginosa*. *Front Biosci* **7**: 354–61

Stephens P, Wall IB, Wilson MJ, et al (2003) Anaerobic cocci populating the deep tissues of chronic wounds impair cellular wound healing responses in vitro. *Br J Dermatol* **148(3)**: 456–66

Key Points

- The conceptual stage of critical colonisation equates to delayed healing in chronic wounds. It is important that clinicians recognise and manage this stage in order to reduce morbidity.
- Various bacterial species are associated with critical colonisation. There are numerous virulence mechanisms which adequately explain this stage.
- Diagnosis is often retrospective as the use of appropriate antimicrobials will often evoke healing.

Trengove NJ, Stacey MC, McGechie D, Stingemore N, Mata S (1994) Qualitative bacteria and chronic leg ulcer healing. Proceedings of the 4th European Wound Management Association Conference on Advances in Wound Management, Copenhagen, Denmark; September 6–9

Trengove NJ, Stacey MC, McGechie DF, et al (1996) Qualitative bacteria and chronic leg ulcer healing. *J Wound Care* **5(6)**: 2772–80

Usher LR, Lawson RA, Geary I, et al (2002) Induction of neutrophil apoptosis by *Pseudomonas aeruginosa* exotoxin pyocyanin; a potential mechanism of persistent infection. *J Immunol* **168(4)**: 1861–68

White RJ (2006) More research is needed before we can accurately define and understand critical colonisation. Letter. *Wounds UK* **2(2)**: 86–8

White R, Cutting K, Kingsley A (2005a) Topical antimicrobials in the control of wound bioburden. *Ostomy/Wound Management* **52(8)**: 26–58

White RJ, Cutting KF, Kingsley A (2005b) Critical colonisation: clinical reality or myth? *Wounds UK* **1(1)**: 94–5

Zychlinsky A, Sansonetti P (1997a) Perspective series: host/pathogen interactions. Apoptosis in bacterial pathogenesis. *J Clin Invest* **100(3)**: 493–5

Zychlinsky A, Sansonetti P (1997b) Apoptosis as a pro-inflammatory event: what can we learn from bacteria-induced cell death? *Trends Microbiol* **5(5)**: 201–4