The pH of wounds during healing and infection: a descriptive literature review

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ABSTRACT

Introduction: Infection is difficult to detect and diagnose in chronic wounds. Misdiagnosis of wound infection risks inappropriate antibiotic prescribing, antibiotic resistance and unnecessary treatment side effects. Alternatively, waiting for the results of microbiological investigations can delay treatment risking spreading or/and systemic infection. There is a need for simple and objective measures of wound infection that provide real-time indication of infection that is accessible to the majority of clinical settings.

Methods: A literature review was conducted that explored existing evidence regarding the association between the pH of a wound and wound healing and infection to determine whether a wound pH scale could be used as a marker of infection.

Results: The literature review found evidence to suggest that wound pH, and specifically an 'Alkaline pH', is conducive to bacterial bio-burden and reflective of the specific physiological processes of the healing cascade.

Conclusions: As such, while pH is not sufficiently understood to be translated into current practice as a metric for infection, the pH scale does have potential as a diagnostic and management indicator and merits exploration.

Keywords: pH, wound, antibiotics, wound infection, clinical indicators.

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INTRODUCTION

Correct diagnosis of an infected chronic wound and appropriate antibiotic use is a contemporary challenge for the wound management field. Factors that need to be considered by the clinician when assessing for wound infection include individual characteristics of the patient, wound characteristics, and the wound environment[1-3]. Current diagnostic techniques for wound infection place emphasis on clinician assessment of infection criteria and sampling of wound surface exudate and material and/or tissue[2,3]. These approaches have benefits and limitations, but neither is able to provide both comprehensive and timely information about wound infection status and the need for, and choice of, antibiotic therapy. Deficiencies of existing wound infection assessment approaches are accentuated by looming concerns relating to antibiotic resistance[1,4,5], deceleration in the discovery of new antibiotics, along with the increasing discovery of antibiotic-resistant organisms[1,3,4,5] and the indiscriminate use of antibiotics in chronic wound care.

Clinical indicators are physical characteristics or symptoms used to inform clinical decision-making in the assessment of wound infection[3,6,7]. In 2005, the European Wound Management Association (EWMA) released a position document specifying and grading the significance of clinical indicators for determining wound infection in different wound aetiologies, with a view to inform accurate and prompt diagnosis of infection and to, subsequently, decrease the indiscriminate use of antibiotics in relation to wound care[6]. Using clinical indicators to identify infection is an approach that is non-invasive, practical and provides an immediate indication of infection. However, the relationship between microbial analysis and clinical indicators as diagnostic measures is inconsistent[7,8]. Limitations of clinical appraisal include misinterpretation of clinical signs especially as this is influenced by clinician experience and training in wound management, and this method additionally fails to indicate antibiotic sensitivity[6,7].

Whilst microbiological analysis is considered an objective determinant of chronic wound infection it may not be the only aspect that needs consideration particularly when the definition of "wound infection" is unclear. While the pathogenic presence of microorganisms contributes to the non-healing state of a wound[9,10], the exact mechanism by which microorganisms negate healing and trigger subsequent negative health events remains unclear. Three hypotheses to illuminate the role of bacteria in wound infection have been proposed including the 'Community Hypothesis' (specific bacterial density results in infection), the 'Specific Bacterial Hypothesis' (specific bacterial pathogens cause infection), and the 'Non-Specific Bacterial Hypothesis' (biofilm causes infection)[9,11-15].

In 1964, Bendly outlined his 'Community Hypothesis' proposing that a relationship existed between increased microbial density and a delay in wound healing[11,13]. Robson and colleagues further defined the hypothesis with a numerical measure of pathogenic bacterial presence, quantifying critical colonisation as >10^5 colony forming units (cfu) per gram of tissue[11,15]. The 'Specific Bacteria Hypothesis' proposed that individual species of bacteria are the causative agents of wound infection[11,15,16] with virulent factors expressed by specific bacteria allowing particular pathogenic bacterial species to outcompete the hosts natural immune system[13]. However, as microorganisms residing in a wound bed can exist in different phenotypes, as either a planktonic state (individual bacteria) or a sessile state (bacteria existing in a community known as a biofilm)[17], conventional microbial detection techniques, used in microbial analysis, favour detection of planktonic bacteria[8,9,18,19]. Planktonic bacteria are located in the tissue, and on the surface of a wound and, therefore, they can be readily identified when using wound swabbing as a method of bacterial analysis, whereas the biofilm cannot[8,18-20]. As such, the presence of particular bacterial species may be underestimated[9,21]. Koch's Postulates denotes that a particular pathogen needs to be present in every case of the disease, that the pathogenic agent can be identified as the cause of disease and cultured, and that if another host is infected with this pathogen that the disease can be cultured. Research to date has not substantiated the proposed relationship between an individual bacterial species and infection which refutes Koch's Postulates[11,21].

The 'Non-Specific Bacteria' hypothesis proposed that the presence of biofilm in a wound inhibits healing and effective removal of the biofilm may allow healing progression[18,21,22]. The biofilm is more commonly present in chronic wounds compared to acute wounds[14]. Bacterial communities, referred to as microcolonies, exist embedded in the extracellular matrix (ECM), which consists of a hydrated extrapolymeric substance (EPS) known as a biofilm[17,21,23-25]. The architecture of the ECM varies in pH, charge, ionic strength and physiochemical structure[23]. The biofilm provides inherent resistance to antimicrobials and host immune responses[11,12,23-25]. Correct identification of the biofilm is difficult for clinicians, as it is not always visible to the human eye, nor can it be detected through wound swabbing techniques[1,17-20]. The role of the biofilm in wound infection has become a more established and integral element within international wound infection guidelines[1,12,18].

Not only is the science of how microorganisms influence wound healing and infection still emerging[1,10], but there are other limitations to the use of objective measures of infection in clinical practice. Wound biopsies are the most accurate means of obtaining tissue samples to identify or quantitate bacteria and to collect samples for determining bacteria sensitivities, particularly in patients who have antibiotic-resistant wounds[20,26]. The accuracy of a wound biopsy, however, may not outweigh the associated risks to the patient[26] due to the increased risk of infection[27]. Wound swabs are less invasive; however, their accuracy compared to wound biopsies is still controversial[20,28]. Furthermore, microbial analyses are not available until post-consultation and as such the practitioner must decide either to treat the wound based on clinical assessment of wound infection and possibly potentiate antibiotic resistance, or alternatively, wait for the microbial results to determine bacteria sensitivities; a delay that may allow the infection to progress.

In short, at present it is confusing for practitioners to definitively determine whether infection is present and if antibiotics are required[1,2,9,15,18,29]. It has been suggested that antibiotic use, as prescribed by general practitioners in relation to chronic wounds, is largely indiscriminate as it most commonly relies on a practitioner's ability to interpret clinical parameters that are not quantitatively defined[1,29,30]. To reduce the indiscriminate over-use of antibiotics, objective measures that yield immediate results to health practitioners and that are accessible from both cost and competence perspectives are required[15,31]. New technologies are in development to provide a real time indication of wound infection (for example, WOUNDCHEK™ Bacterial Status, WOUNDCHEK™ Laboratories). However, even when commercially available, these tools may not be accessible in all clinical settings.

An increase in the pH of infected wounds may influence the bacterial virulence as well as bacterial growth[32,33]. Given the relationship between pH and microorganism growth, it is proposed that the pH value of a wound is a neglected parameter in clinical practice. Wound pH and the measurement of it in clinical practice may have the potential to improve the accuracy of infection diagnosis and be used as a predictor of wound progression. Wound pH may be used to indicate the likelihood of healing either to supplement current subjective assessment with a real time objective, measure either as a standalone or as a part of more comprehensive infection diagnostic tool that is in development. Furthermore, pH monitoring is an inexpensive, brief, and non-invasive test that clinicians with limited instruction could perform, and it provides a rapid report of the wound's status. The purpose of this paper was to conduct a literature review to explore existing knowledge about the pH of a wound, wound healing, and wound infection.

METHOD

A descriptive literature review was adopted as the method for this review. As described by Booth and colleagues (2009) the descriptive literature review is one of 14 types of reviews[34]. This particular method was chosen as this was an area of enquiry where it was anticipated that there would be limited literature and what evidence was available would be derived from a low level of evidence[34]. Publications for this descriptive literature review were predominantly obtained from the multiple online databases including Medline, CINAHL, PubMed, ProQuest Central, Elsevier SD Freedom Collection, MA Healthcare InterNurse, Wiley Online Library, Highwire Press American Society for Microbiology. The search was supplemented by targeted review of key associations including the European Wound Management Association, Wounds Australia, International Wound Infection Institute, the World Health Organization, and the Cochrane Library. Principle search terms included 'chronic wounds' 'diagnostic,' 'pH,' 'biofilm,' 'infection,' 'antibiotics,' 'debridement,' 'haemostasis,' 'indicators of infection', 'wound repair,' and 'healing cascade'. To refine the literature search, the following limits were applied to the search; peer reviewed, publication date 2006 to present, and English language. Boolean Operators such as 'AND' and 'OR' were used where applicable. The reference lists of relevant articles were hand-searched to identify additional articles related to the literature review foci that did not appear in the database search.

RESULTS

pH is a logarithmic measure ranging from 1 to 14 in value, of hydrogen ions, which indicates acidity (<7), neutral (7) or basicity (>7)[35]. The skin of a person exists at a pH range of 4.2–5.6[35]. This acidic range provides a contentious antimicrobial defence discouraging the overgrowth of bacteria[29,36-38]. The skin is also a physical barrier to infection; however, upon perforation the exposed underlying tissue exists at a pH milieu of 7.4[32,35,36,39]. The pH of a wound can influence susceptibility to infection, antimicrobial activity, growth and toxicity, modulate by-product production and influence biochemical process within the wound[29,35,36,40-44].

pH AND BACTERIA IN WOUNDS

The pH of a wound can support or reduce microorganism invasion and proliferation[36]. The minimum pH at which *Escherichia coli, Pseudomonas aeruginosa* and *Streptococcus pyogenes* can grow is 4.3, 4.4 and 4.5 respectively[40]. Ono and colleagues determined that the presence of *Staphylococcus aureus* and *Staphylococcus epidermis* were cultured from an infected burns wounds with increased pH[29]. Furthermore, the transcription level of bacterial DNA for *S. aureus*, and its growth, is accelerated *in vitro* and more pronounced in an alkaline environment[32,33]. In 2005, Harjai and colleagues studied the effect of pH on the production of virulent in *Pseudomonas aeruginosa* finding that an alkaline environment increased bacterial growth and virulence as more by-products such as alginate and proteinase, were manufactured at pH 8 versus pH 5[45]. Bi-products of bacteria such as ammonia can impair oxygenation of wound tissue and through necrosis can promote a more alkaline wound environment[32].

The effect of pH (5.5, 7.5, 8.5) on the biofilm of the various bacterial species *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Vibrio cholera (*non-O1 and O1*)* was examined by Hostacká and colleagues (2010) using a crystal violet test*.* Their research demonstrated, *in vitro*, that all strains of bacteria had increased biofilm growth in environments with increased alkaline pH[38]. Once constructed it has been identified that biofilms can survive in pH ranges that would normally be inhibitory to growth under planktonic conditions[25,32,46].

In turn, the growth of bacteria in a wound further perpetuates its alkalinisation[39]. The pH value may be used not just to determine infection, but to manipulate bacterial growth promoting and predicting wound healing[29,38,47]**.**

The pH of a wound can additionally influence the effectiveness of antibiotics and antiseptics[22,31,39]. The pH may modulate the effectiveness and success of antibiotic performance or potentially alter the metabolic state of bacteria[22], allowing bacterial growth and acquired resistance to occur. For example, gentamycin and silver antiseptic's effectiveness is reduced in acidic environments[13]. Comparatively, beta lactams, quinolones, chlorhexidine, quaternary ammonium compounds are more active in alkaline environments. Biofilms require a multimodal treatment as no single antibiotic agent has been determined to be as effective[22]. Biofilms exhibit an antibiotic tolerance compared to planktonic bacteria[12,23,31].

pH AND WOUNDS

Open wounds characteristically have a neutral to alkaline pH existing in the pH range of 6.5 to 8.5 while chronic wounds exist at a range of 7.2 to 8.9[32]. Monitoring pH can help predict the progression of wound healing as the pH of a wound can be indicative of the biochemical processes of healing[36,39] and the pH of the tissue in the wound bed will, in turn, fluctuate[32,39]. Initially, the wound undergoes acidosis, with increased lactic acid and oxygen in the wound decreasing the pH[48]. Acidosis of the wound is required for the proliferation of fibroblasts, DNA cell synthesis, oxygenation, collagen formation, angiogenesis and macrophage activity[39,49]. Keratinocyte differentiation may be induced at a decreased pH[43]. Acidic wound fluid has been associated with more rapid wound healing[36]; however, chronic wounds exist in an alkaline environment. An alkaline wound environment impairs the healing and immunological response by promoting bacterial growth increasing proteolytic activity, inhibiting fibroblasts and decreasing oxygen supply[36,39,42]. An understanding of pH and refinement of how to use this metric could help clinicians determine the likelihood of wound healing in the future[29].

In chronic wounds, catabolic processes predominate causing an interruption of the biochemical process that facilitates wound healing. Decreased oxygen tension lowers the pH which affects angiogenesis, immunological activity and collagen formation[32,42]. Cells that operate in anaerobic conditions produce lactate which contributes to the alkaline milieu of the wound[32]. Chronic wounds have increased protease levels that can be influenced by the pH milieu of the wound; decreased pH is associated with a decreased proteolytic activity[42]. In healthy wounds, matrix metalloproteinases (MMPs) remodel the extracellular matrix (ECM); however, elevated levels of MMPs enzymes are detrimental to wound healing[22,39,50]. Acidic environment decreases MMPs, thus reducing corruption to the extracellular matrix[39,42]. Given that an alkaline wound pH is associated with reduced wound healing prognosis, modulating pH to a more acidic environment has been observed to increase the rate of wound healing[37,40-43]. Altering the wound surface pH stimulates wound healing by promoting angiogenesis, collagen formation and the immunological response, specifically, macrophage recruitment[32]. To illustrate this point, in 2014, Milne and Connolly, conducted an *in vitro* investigation of four wound dressing types with varied acidic pH levels (Manuka honey dressing, sodium carboxymethylcellulose hydrofibre, polyhydrated ionogen-coated polymer mesh and protease modulating collagen cellulose) on wound healing and determined that the most acidic treatments had the most promising healing effect on the simulated wound[41]. Nagoba and colleagues (2011) conducted a prospective study assessing the efficacy of citric acid 3% ointment as a sole antimicrobial agent in surgical patients. The ointment was applied daily, with adverse effects recorded in only 1.43% of cases and were limited to mild irritation 2–3 minutes post application of the ointment. Bacterial cultures were collected from the wounds of 70 patients, with 96 isolates identified; sensitivities to antimicrobial agents were examined, bacterial resistance was present. Comparatively, all bacteria was sensitive to the citric acid with *P. aeruginosa*, the most commonly identified pathogen, the most susceptible to the citric acid formulation[47]. Agents such as citric acid, which acidify a wound, can yield successful treatment results, and should be considered as routine agent in wounds[47,51,52]. Citric acid is thought to assist "*wound healing by increased fibroblast proliferation and probably increasing local oxygen concentration and reducing microbial growth and virulence*"[52].

DISCUSSION

Globally, infections that formerly responded to antibiotics are developing resistance to previously successful therapy[4,6,31,53-55]. Unrestricted antibiotic use has been associated with an increased prevalence of antibiotic-resistant pathogens[31,53,54] and is resulting in an evolutionary selection pressure favouring bacteria with advantageous genetic mutations or acquired resistance through antibiotic-resistant DNA transfer[5,53,54]. As antibiotic resistance continues to progress, it is clear that a more cautious use of this resource is required. The discovery of antibiotics revolutionised medical practice and without such therapy the future of modern medicine becomes unknown[56]. The discovery of new antibiotics has slowed, with an increasing trend in the discovery of antibiotic-resistant organisms[53,55,56]. Practitioners are now observing bacteria that are resistant to all available antibiotics[57]. The only sustainable solution is to limit the overuse and misuse of antibiotics and it is crucial that practitioners are vigilant in using validated diagnostic criterion when choosing to prescribe antibiotics and implement the use of scientific evidence to support clinical practice[4,5,15,53].

The judicious use of antibiotics in chronic wound care requires stewardship that can be gained through accurate and accessible diagnostic tools. In this field, there is a lack of evidence concerning effectiveness, optimal regimens or clinical interventions for antibiotic treatment of wound infections[6,15]. Antibiotic use in chronic wound care is largely indiscriminate as it primarily relies on a practitioner's ability to interpret parameters that are not quantitatively defined[15,30]. Antibiotics are a feature of the management of chronic wounds and these patients receive significantly more antibiotics (systemic and topical) than age- and sex-matched patients without chronic wounds[15]. Current guidelines for antibiotic prescribing for chronic wounds are often based on expert opinion rather than scientific data. To reduce the indiscriminate overuse of antibiotics, objective measures that yield immediate results to health practitioners and that are accessible from both a cost and competence perspective are required[2,15]. The pH value of a wound is a neglected parameter in clinical practice that has the potential to improve the accuracy of infection diagnosis[40].

This literature review sought to describe existing knowledge about the role of pH on wound healing and infection. It identified that chronic or infected wounds exist at a more alkaline pH environment which is more conducive to bacterial burden. A rise in bacterial colonisation will increase the alkalinity of a wound to further optimise bacterial growth[36]. Furthermore, the presence of bacteria which alkalinise a wound may result in inflammation and play a part in synthesising a chronic wound[32]. A subsequent effect of the wound pH on the effectiveness of antibiotics and antiseptics has also been described[39]. Thus, wound pH is implicated in creating wound environments that are favourable for bioburden that delays healing and leads to infection, as a consequence of bioburden, and as a barrier to effective treatment. pH would appear to be a relevant metric of wound healing. Thus, wound pH is implicated in creating wound environments that are favourable for bioburden that delays healing and leads to infection.

Although increasing bacterial burden is associated with an increasing pH, the exact relationship between pH and the degree of contamination (Community Hypothesis) or species of bacterial contamination (Specific Bacterial Hypothesis), or association with the biofilm (Non Specific Bacterial Hypothesis) is yet to be established[1,9,12,13,39]. Quantitatively defining this relationship would provide an objective measure for clinicians to indicate when infection is present. To accurately define the relationship between pH and the bacteria profile, the term 'infection' needs to be more precisely defined. The influence of pH as identified in this review could be interpreted as supporting each of these hypotheses in turn. Both the Community and Specifically Bacterial Hypotheses could be supported as chronic or infected wounds exist at a more alkaline pH (≥7.3 pH)[30], an environment that is more conducive to bacterial burden, thus enabling a degree of contamination. Although as an alkaline milieu is preferred by most of the specific bacteria noted in the sourced literature, it cannot be discounted that wound infection is a direct consequence of specific bacteria.

The pH of a wound is also involved in the development and management of a biofilm. This is evidenced by identifying that bacteria increased biofilm growth in an alkaline environment[37]. It has also been shown that once established biofilms become resistant to fluctuations in pH[31,32] they can survive in pH ranges that would normally be inhibitory to growth under planktonic conditions[32,41].

The fortitude of a biofilm to changes in pH parallels the response of the biofilm to antibiotic and antimicrobial interventions and given this association would also support the Non-Specific Bacterial Hypothesis regarding wound infection.

The lack of published evidence pertaining to wound healing and infection and pH is a limitation of the current literature review. Additionally, there is the possibility that literature reporting on pH may not have been identified if pH was not included as a key word. Further research should be dedicated to defining the relationship between bacterial species, biofilm and bacterial density with pH, as the association has the potential to contribute to diagnostic measures in wound infection. pH measurement offers additional diagnostic advantages over clinical indicators as clinical indicators can only show when infection is present, comparatively, the pH milieu of the wound increases as infection manifestation begins[29].

Pending further information of how pH could be an accurate marker of infection to guide practice, the incorporation of pH into practice would present the majority of clinicians and clinical environments with a measure that is objective, reproducible, and is not influenced by clinician experience or expertise[36]. Additionally, the test is inexpensive, minimally invasive and produces immediate results that are not available with wound swabbing or biopsies[36]. Wound pH would supplement the clinician's interpretation of clinical indicators for infection and assist with assessing wound healing conditions more generally. Given evidence that pH affects the pathophysiology, microbiology and immunology of wound healing, it remains a neglected parameter in current practice, that has the potential to become a valuable diagnostic tool[32,36,40]; an area that warrants further investigation[9]. Further research would not only reveal the potential for pH to be a standalone or supplementary tool in a suite of objective measures into the future, but could provide further information about how microorganisms influence wound healing and infection.

LIMITATIONS

This study investigated the topic 'pH and wound care during infection and healing' using a descriptive literature review. The limitations of this method are there can potentially be bias to omit or select literature that supports a particular view[34]. The quality of the studies was not formally assessed and presented to the reader, like that of a systematic review. A challenge associated with this literature review topic is the difficulty to isolate relevant publications using the search terms alone and reflects the burgeoning nature of this topic area in published form. A meta-analysis could not be undertaken, as there were not a sufficient number of similar studies in this research area.

CONCLUSION

To provide a more holistic approach to wound care, assessment diagnostic tools require improvement. In clinical practice, the pH of a wound is not a parameter considered in the assessment of chronic wounds healing. Wound pH impacts the effectiveness of antibiotics in a wound bed and its modulation could improve patient outcomes. Numerous researchers have, however, suggested pH has the potential to become a valuable diagnostic tool[29,32,36,39,40]. Research has determined that alkaline wound pH is conducive to bacterial bioburden and reflective of the specific physiological processes of the healing cascade[32,39]. The potential of pH as a diagnostic indicator of infection therefore requires exploration[32,40]. The association between pH with both bacteria profile and the healing cascade needs to be defined for practitioners to know what pH results are an indicator of and to determine if antibiotics are indicated or if a wound in its present state is unlikely to heal. Explicitly defining these relationships would allow pH to become an objective measure for use practice. Further research is required exploring these relationships. Using wound pH as a diagnostic indicator has the potential to enhance clinical success, aiding the decision making process of clinicians and improving wound management.

REFERENCES

1. Wounds International 2016. Wound Infection in Clinical Practice: Principles of Best Practice. International Wound Infection Institute (IWII). 2016.
2. Woo KY. Physicians' knowledge and attitudes in the management of wound infection. Int Wound J 2016;13(5):600.
3. Swanson T, Asimus M, McGuiness B. Wound management for the advanced practitioner. Melbourne, Australia: IP Communications; 2014.
4. World Health Organization. Antibiotic resistance [Fact-Sheet]. World Health Organization; 2016 [updated October 2016; cited 2016 November 2016]. Available from: http://www.who.int/mediacentre/factsheets/antibiotic-resistance/en/.
5. Walsh C. Molecular mechanisms that confer antibacterial drug resistance. Nature 2000;406(6797):775–81.
6. European Wound Management Association (EWMA). Position document: identifying criteria for wound infection. EWMA; 2005.
7. Gardner S, Frantz R, Doebbeling B. The validity of the clinical signs and symptoms used to identify localized chronic wound infection. Wound Repair Regen 2001;9(3):178–86.
8. Miller C, Carville K, Newall N, Kapp S, Lewin G, Karimi L *et al.* Assessing bacterial burden in wounds: comparing clinical observation and wound swabs. Int Wound J 2011;8(1):45–55.
9. Kirketerp-Moller K, Jensen P, Fazli M, Madsen K, Pedersen J, Moser C *et al.* Distribution, organization, and ecology of bacteria in chronic wounds. J Clin Microbiol 2008;46(8):2717–22.
10. Wolcott R, Stanford N, Gabrilska R, Oates J, Wilkinson J, Rumbaugh K. Microbiota is a primary cause of pathogenesis of chronic wounds. J Wound Care 2016;25(10).
11. Percival S, Thomas J, Williams D. Biofilms and bacterial imbalances in chronic wounds: anti-Koch. Int Wound J 2010;7(3):169–75.
12. Bjarnsholt T, Kirketerp-møller K, Jensen PØ, Madsen KG, Phipps R, Krogfelt K *et al.* Why chronic wounds will not heal: a novel hypothesis. Wound Repair Regen 2008;16(1):2–10.
13. Bowler P, Duerden B, Armstrong D. Wound microbiology and associated approaches to wound management. Clin Microbiol Rev 2001;14(2):244–69.
14. James G, Swogger E, Wolcott R, deLancey-Pulcini E, Secor P, Sestrich J *et al.* Biofilms in chronic wounds. Wound Repair Regen 2008;16(1):37.
15. Howell-Jones R, Wilson M, Hill K, Howard A, Price P, Thomas D. A review of the microbiology, antibiotic usage and resistance in chronic skin wounds. J Antimicrob Chemother 2005;55:143–9.
16. Robson MC, Duke WF, Krizek TJ. Rapid bacterial screening in the treatment of civilian wounds. J Surg Res 1973;14(5):426–30.
17. Davis S, Serralta V, Harrison-Balestra C, Cazzaniga A, Mertz P. Lifestyles of bacteria in wounds: Presence of biofilms? J Invest Dermatol 2001;117(2):436–.
18. Costerton W, Veeh R, Shirtliff M, Pasmore M, Post C, Ehrlich G. The application of biofilm science to the study and control of chronic bacterial infections. J Clin Invest 2003;112(10):1466.
19. Wolcott R, Rhoads D. A study of biofilm-based wound management in subjects with critical limb ischaemia. J Wound Care 2008;17(4):145.
20. Copeland-Halperin LR, Kaminsky AJ, Bluefeld N, Miraliakbari R. Sample procurement for cultures of infected wounds: a systematic review. J Wound Care 2016;25(4):S4.
21. Dowd S, Wolcott RD, Sun Y, McKeehan T, Smith E, Rhoads D. Polymicrobial nature of chronic diabetic foot ulcer biofilm infections determined using bacterial Tag Encoded FLX Amplicon Pyrosequencing (bTEFAP). PLoS One 2008;3(10).
22. Percival S, Finnegan S, Donelli G, Vuotto C, Rimmer S, Lipsky BA. Antiseptics for treating infected wounds: Efficacy on biofilms and effect of pH. Crit Rev Microbiol 2014:1–17.
23. Percival S, Hill K, Malic S, Thomas D, Williams D. Antimicrobial tolerance and the significance of persister cells in recalcitrant chronic wound biofilms. Wound Repair Regen 2011;19(1):1–9.
24. Davies D. Understanding biofilm resistance to antibacterial agents. Nat Rev Drug Discov 2003;2(2):114–22.
25. Davis SC, Ricotti C, Cazzaniga A, Welsh E, Eaglstein W, Mertz P. Microscopic and physiologic evidence for biofilm-associated wound colonization *in vivo*. Wound Repair Regen 2008;16(1):23–9.
26. Davies C, Hill K, Newcombe R, Stephens P, Wilson M, Harding K *et al.* A prospective study of the microbiology of chronic venous leg ulcers to reevaluate the clinical predictive value of tissue biopsies and swabs. Wound Repair Regen 2007;15(1):17–22.
27. Alavi A, Niakosari F, Sibbald R. When and how to perform a biopsy on a chronic wound. Adv Skin Wound Care 2010;23(3):141–2.
28. Slater R, Lazarovitch T, Boldur I, Ramot Y, Buchs A, Weiss M *et al.* Swab cultures accurately identify bacterial pathogens in diabetic foot wounds not involving bone. Diabet Med 2004;21(7):705–9.
29. Ono S, Imai R, Ida Y, Shibata D, Komiya T, Matsumura H. Increased wound pH as an indicator of local wound infection in second degree burns. Burns 2014;41(4):820–24.
30. Hardy-Holbrook R, Aristidi S, Chandnani V, DeWindt D, Dinh K. Antibiotic resistance and prescribing in Australia: current attitudes and practice of GPs. Healthc Infect 2013;18(4):147–51.
31. Thomas J, Linton S, Corum L, Slone W, Okel T, Percival SL. The affect of pH and bacterial phenotypic state on antibiotic efficacy. Int Wound J 2012;9(4):428.
32. Percival S, Woods EJ, McCarty S, Hunt JA. The effects of pH on wound healing, biofilms, and antimicrobial efficacy. Wound Repair Regen 2014.
33. Weinrick B, Dunman PM, McAleese F *et al.* Effect of mild acid on gene expression in *Staphylococcus aureus*. J Bacteriol 2004;186(24):8407.
34. Grant MJ, Booth A. A typology of reviews: an analysis of 14 review types and associated methodologies. Health Info Libr J 2009;26:91–108.
35. Hampton S. Understanding the pH balance in wound healing. J Community Nurs 2008;22(5):34.
36. Shukla V, Shukla D, Tiwary S, Agrawal S, Rastogi A. Evaluation of pH measurement as a method of wound assessment. J Wound Care 2007;16(7):291.
37. Schmid-Wendtner M, Korting H. The pH of the skin surface and its impact on the barrier function. Skin Pharmacol Physiol 2006;19(6):296–302.
38. Hoštacká A, Čižnár I, Štefkovičová M. Temperature and pH affect the production of bacterial biofilm. Folia Microbiol 2010;55(1):75–8.
39. Schneider L, Korber A, Grabbe S, Dissemond J. Influence of pH on wound-healing: a new perspective for wound-therapy? Arch Dermatol Res 2007;298:413–20.
40. Gethin GT, Cowman S, Conroy RM. The impact of Manuka honey dressings on the surface pH of chronic wounds. (Report). Int Wound J 2008;5(2):185.
41. Milne SD, Connolly P. The influence of different dressings on the pH of the wound environment. J Wound Care 2014;23(2):53–7.
42. Greener B, Hughes A, Bannister N, Douglass J. Proteases and pH in chronic wounds. J Wound Care 2005;14(2):59.
43. Sharpe JR, Harris KL, Jubin K, Bainbridge NJ, Jordan NR. The effect of pH in modulating skin cell behaviour. Br J Dermatol 2009;161(3):671–3.

44. Basavraj S Nagoba, Namdev M Suryawanshi, Bharat Wadher, Sohan Selkar. Acidic environment and wound healing: a review. Wounds 2015;27(1).
45. Harjai K, Khandwaha R, Mittal R, Yadav V, Gupta V, Sharma S. Effect of pH on production of virulence factors by biofilm cells of *Pseudomonas aeruginosa*. Folia Microbiol 2005;50(2):99–102.
46. Percival SL, Finnegan S, Donelli G, Vuotto C, Rimmer S, Lipsky BA. Antiseptics for treating infected wounds: Efficacy on biofilms and effect of pH. Crit Rev Microbiol 2016;42(2):293.
47. Nagoba B, Raju R, Wadher B *et al.* Citric acid treatment of surgical site infections: a prospective open study. Wound Practice & Research 2011;19(2):82–6.
48. Khan MA, Ansari U, Ali MN. Real-time wound management through integrated pH sensors: a review. Bradford: Emerald Group Publishing, Limited; 2015, pp. 183–9.
49. Liu Y, Kalen A, Risto O, Wahlstrom O. Fibroblast proliferation due to exposure to a platelet concentrate *in vitro* is pH dependent. Wound Repair Regen 2002;10(5):336–40.
50. Sibbald RG, Woo K. The biology of chronic foot ulcers in persons with diabetes. Diabetes Metab Res Rev 2008;24:S25–S30.
51. Nagoba BS, Aghav VV, Wadher BJ, Rao A, Selkar SP. The use of citric acid for the treatment of chronic non-healing sinus. Iran J Med Sci 2011;36(1):63–4.
52. Prabhu V, Prasadi S, Pawar V, Shivani A, Gore A. Does wound pH modulation with 3% citric acid solution dressing help in wound healing: A pilot study. Saudi Surg J 2014;2(2):38.
53. MacGowan A, Macnaughton E. Antibiotic resistance. Nurs Stand 2014;29(10):18.
54. Bartlett JG, Gilbert DN, Spellberg B. Seven ways to preserve the miracle of antibiotics. Clin Infect Dis 2013;56(10):1445.
55. Challenges of antibacterial discovery. Clin Microbiol Rev 2011;24(1):71.
56. Spellberg B, Bartlett J, Wunderink R, Gilbert D. Novel approaches are needed to develop tomorrow's antibacterial therapies. Am J Respir Crit Care Med 2015;191(2):135–40.
57. Carlet J. Antibiotic resistance: Protecting antibiotics — the declaration of the world alliance against antibiotic resistance. Indian J Crit Care Med 2014;18(10):643–5.