PERSPECTIVE ARTICLE

The effects of pH on wound healing, biofilms, and antimicrobial efficacy

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ABSTRACT

It is known that pH has a role to play in wound healing. In particular, pH has been shown to affect matrix metalloproteinase activity, tissue inhibitors of matrix metalloproteinases activity, fibroblast activity, keratinocyte proliferation, microbial proliferation, and also immunological responses in a wound; the patient's defense mechanisms change the local pH of a wound to effect microorganism invasion and proliferation; this pH change has been found to affect the performance of antimicrobials, and therefore the efficacy in biological environments directly relevant to wound healing. Based on the available body of scientific evidence to date, it is clear that pH has a role to play in both the healing of and treatment of chronic and acute wounds. It is the purpose of this review to evaluate the published knowledge base that concerns the effect of pH changes, the role it plays in wound healing and biofilm formation, and how it can affect treatment efficacy and wound management strategies.

Cutaneous wound healing involves complex independent and dependent pathways which employ numerous cell lineages, tissues, and intrinsic and extrinsic mediators.[1,2] If wound healing continues through the "normal" series of biological events, then effective healing occurs, and homeostasis is restored. This normal series of biological events includes hemostasis, inflammation, proliferation, and remodeling.[2,3] However, any interruption or interference to these pathways results in a nonhealing or "stalled" wound, rendering the wound chronic. Conditions within a chronic wound are in a chaotic biological flux that will inevitably increase the wound's propensity to infection. Infection itself may halt the healing process, holding the wound in the inflammatory phase of healing.[4]

Many factors are known to have an effect on wound healing, and one area of particular importance is intracellular and extracellular pH. Cellular processes including enzyme activity, macromolecular synthesis, transport of metabolites, and cell cycle progression are dependent on both intracellular and extracellular pH.[5] Schneider and colleagues[6] provided a detailed overview of the relationship between pH and wound healing and noted the complexity of pH changes in wound healing. They demonstrated that pH is an important contributing factor in the healing process, and in particular, different pH ranges are required for the various phases of healing. Kaufman and Berger[7] also highlighted the links between wound healing and topical pH and concluded that wound healing could be controlled, in part, by changing pH levels. In terms of pH as a therapeutic target, it has been demonstrated that wound healing occurs most effectively at low pH, whereas alkaline wound environments have been linked predominately to chronic wounds.[2]

Many healing processes are affected by changes in pH including angiogenesis, collagen formation, and macrophage activity.[2] A change in pH has also been shown to influence the toxicity of bacterial end products and affect enzyme activity.[2] In particular, the matrix metalloproteinases (MMPs), which are important for wound healing and extracellular matrix remodeling, have been shown to be sensitive to small fluctuations in pH.[2,8–10] Studies have also reported that variations in pH may affect wound closure, graft take, microbial infection rates, bacterial virulence, and biofilm formation.[11,12]

In light of the importance of pH to wound healing, it has been proposed that monitoring the surface pH of wounds may

ATR	Acid tolerance response
IL	Interleukin
MDT	Maggot debridement therapy
MIC	Minimal inhibitory concentration
MMP	Matrix metalloproteinase
pHi	Intracellular pH
PMN	Polymorphonuclearcyte
SC	Stratum corneum
SP	Serine protease
SpeB	Streptococcal pyrogenic exotoxin B
TEWL	Transepidermal water loss
TNF-α	Tumor necrosis factor-α
ZOI	Zone of inhibition

be helpful in guiding management practices and determining the most effective treatment strategies.[2] However, in order to ensure treatment strategies are more effective, monitoring of the wound status on a weekly basis would be required.[13] Given that current wound management involves mainly subjective assessment of wound progression, objective measurements of healing states may provide a more accurate way of assessing the condition of the wound throughout treatment and may prove useful in routine clinical wound care settings.[2] In addition, a number of topical preparations and dressings have been developed with an aim to control or alter wound surface pH. Some examples of pH-modulating products include occlusive dressings (which prevent the loss of carbon dioxide from the tissue, thus preventing respiratory alkalosis),[2,14] honey,[15] and acidified nitrate creams.[16,17] Acidification of the wound bed has been found to both aid in the healing of the wound and also help control polymicrobial infections.[2] In the case of honey, studies have documented several therapeutic effects which improve wound healing including antimicrobial activity and ability to stimulate tissue growth.[18] Evidence suggests that a key factor in the efficacy of honey is its low pH (honey typically has a pH from 3 to 4).[19] Likewise, nitric oxide has antimicrobial effects; it is continually released from normal skin and protects the skin from infection.[20] Indeed, acidified nitrite creams have been shown to be effective both in in vitro investigations and in clinical trials in a range of wound types.[16,17,20] Experimental studies have shown that the microbiocidal effects of nitrite are dependent on its acidification.[16] Acidification of the wound bed has been shown to promote healing by causing more oxygen to be released from hemoglobin which in turn reduces the hydrostatic pressure in the interstitial fluid, thus allowing improved circulation in the tissues.[19]

Although pH is known to affect many of the fundamental physiological and biochemical processes involved in wound remodeling, other factors may moderate these effects. For example, it is well known that oxygen tension can affect tissue repair given its requirement in fibroblast growth and collagen synthesis[21,22] with ischemic wounds notably having poor healing qualities.[23] As oxygen can alter pH, oxygen tension is also a confounding factor and an important consideration when assessing the role of pH in wound healing.[2,24] Other factors can also influence tissue pH, for instance, bacterial by-products including ammonia which raise tissue pH, resulting in an ideal environment for further bacterial proliferation,[21] causing a positive feedback mechanism.

It is the purpose of this review to evaluate the available literature to determine the role of pH in wound healing, biofilm formation, microbial virulence, immune response, and the effect it can therefore have on the treatment and management strategies presently being employed for effective wound healing.

pH AND WOUND HEALING

Wound healing is a complex process which can affect or be affected by changes in pH at every phase.[8,21,25,26] Early studies have reported that the pH of a chronic wound exists in the range of 7.15–8.9.[8,27,28] Roberts and colleagues[29] demonstrated that wounds with a high alkaline pH had a lower healing rate when compared with wounds with a pH closer to neutral. Further work by Hoffman and colleagues reported that within alkaline conditions, wound healing progression decreased.[30] More recent reviews by Gethin[2] reported further on the role pH plays in wound healing and provided more evidence that the acute and chronic wound environment progresses from an alkaline state to a neutral and then acidic state during healing. As such, numerous researchers have suggested that pH could be used as an indicator of wound healing, providing a useful diagnostic tool.[8,21,25,29]

CLINICAL EVIDENCE FOR THE ROLE OF pH IN WOUND HEALING

Dissemond and colleagues[25] described variations in pH values measured in 39 different patients with chronic wounds over a 12-month period. The study measured a total of 247 pH values of chronic wounds with varying etiology and found that chronic wound pH values ranged from 5.45 to 8.65 with the mean at 7.42. The authors emphasized how fluctuations in pH directly or indirectly influenced the outcome of chronic wound healing. Greener and colleagues[31] observed that, in order to test their hypothesis that pH had an effect on wound protease activity, a suitable model was needed which could accurately assess protease activity and corresponding pH levels in undiluted, unbuffered wound fluid samples. The group developed an in vitro test using a film consisting of a 7-μm thick cross-linked gelatin coating mounted on polyester film and used this to quantitatively determine the volume of protease degradation and thus level of activity in wound fluid sample. Following this study, it was concluded that decreasing the pH level of wounds could be an effective way of reducing protease activity as a mechanism to promote healing.

Studies that have focused on the evaluation of topical agents that modify wound pH have provided evidence for the benefits in terms of wound management. A blinded study by Kaufman et al.[32] assessed the effects of three buffered solutions with pH values of 3.5, 7.42, and 8.5 on the healing rates of deep partial thickness burns for 21 days in guinea pigs. The topical acidification of these wounds promoted epithelialization and increased healing rates; the rate of epithelialization of the wounded area was significantly greater ($p < 0.001$) in those wounds treated with the pH 3.5 solution. Cadesorb (Smith & Nephew, Hull, United Kingdom) is a commercially available ointment which modulates protease activity by controlling local wound pH. The use of this product was shown to have a positive effect on the healing of 10 cases of leg and foot ulcers and was shown to effectively reduce the pH of the wound surface and also minimize patient pain.[33]

Roberts et al.[34] investigated the significance of surface pH and temperature in venous ulcers. This randomized study of 25 patients revealed that the mean wound surface pH of the healing wounds was 6.91 compared with a mean pH value of 7.42 for the nonhealing wounds. It was concluded that low pH and higher wound temperature were conducive to healing. Similarly, Shukla et al.[35] assessed the relationship between wound pH and the state of wound healing in 50 patients with acute or chronic wounds. Wound pH (using litmus paper strips) together with other parameters including wound condition, exudate, and microbiology was measured and recorded weekly. Overall, results showed that at baseline, the pH of the majority of wounds studied was greater than 8.5, yet as the wounds showed signs of healing, the pH reduced to less than 8.0. The authors concluded that measurements of wound pH

can be performed efficiently and are noninvasive, causing no discomfort to the patient, and the change in pH can help predict prognosis.

Leveen et al.[21] suggested that oxyhemaglobin releases more oxygen in an acid environment and noted that bacteria that produced ammonia impaired oxygenation of the wound because of an increase in pH. They also reported that the pH of the solution was a significant and critical factor in the toxicity of ammonia. In addition, Wilson et al.[27] supported the prolonged chemical acidification of varicose ulcer surfaces and proposed that the positive effect on healing was brought about by an influence on oxygen levels.

Work carried out by Shi et al.[36] addressed two key roles of wound pH. Firstly, the study sought to understand the influence of pH on *Clostridium* collagenase activity via a collagen-based artificial wound eschar in vitro model in terms of debridement efficacy of bacterial collagenase under varying pH values. Secondly, the influence of bacterial contamination on pH in vivo was investigated. Wound fluid of a contaminated pig wound model was used to examine pH during healing. This wound model was challenged with a bacterial load including *Pseudomonas aeruginosa*, coagulase-negative *Staphylococci*, and *Fusobacterium* sp. to track the pH of wounds in relation to bacterial load. Results indicated that pH levels in the wound fluid were all above neutral, and collagenase activity reached its peak at a pH value around 8.5. Although variations were seen in terms of bacterial load between bacterial species, it was found that no significant change in the level of bacterial bioburden occurred. Although the wounds were alkaline during the first 10 days of healing, pH ultimately increased during healing, demonstrating the resistance of these bacteria and collagenase activity to the changing wound environment.

Clearance of necrotic tissue, exudates, foreign material, and microorganisms by applying sterile fly larvae, typically of *Lucilia sericata*, is known as maggot debridement therapy (MDT) and has been shown to provide rapid and effective wound debridement. An early study carried out by Robinson in 1940[37] observed that ammonium bicarbonate was secreted as a metabolic product by surgical maggots. This was concluded to be a factor affecting the pH of MDT-treated wounds and was considered to contribute toward their clinical effectiveness. A more recent study showed that the enzyme-rich secretions of maggots function optimally at around pH 8–8.5; therefore, any changes in pH because of the wound environment could impact the activity of larval secretions as well as native enzymes.[38]

EFFECT OF pH ON SKIN CELLS IN WOUND HEALING

Sharpe et al.[39] reported on the effect of pH on cell behavior and wound healing. The study investigated the effect of pH on the attachment, proliferation, and migration of keratinocytes and fibroblasts using in vitro and ex vivo skin cell models. Additionally, the effect of pH on keratinocyte differentiation was measured by the expression of cytokeratins, the proteins of keratin intermediate filaments found between epithelial cells, in particular cytokeratins 1 and 5. It was concluded that a differentiated keratinocyte phenotype is promoted at low pH values. Furthermore, an optimal pH for both keratinocyte and fibroblast proliferation was proposed to be between pH 7.2 and 8.3, whereas the optimal pH for growth from ex vivo skin explants was 8.4, demonstrating that skin cells and explants proliferate and migrate at pH values higher than physiological pH.

Rubin[40] described how pH influences cell migration and how cell density creates changes in pH suited to healing. It was found that sparse chick embryo cell cultures were much less sensitive to pH reduction in terms of cellular multiplication when compared with dense cultures. Wounding experiments revealed similar results, with low pH inhibiting cell migration. However, those cells which migrated into the wounded area multiplied as rapidly at low pH as at high pH, demonstrating the combined effects of pH and cell density. Interestingly, Rous sarcoma cells were also less sensitive to pH changes at high densities compared with normal cells at the same density, but they were more sensitive as compared with sparse normal cultures. Similarly, Taylor and Hodson[41] presented data suggesting a role for pH in cell regulation; in this case, normal growth of human tumor cells occurred at pH values between 7.2 and 6.8.

Liu and colleagues[42] highlighted the indirect effects of pH on fibroblast proliferation. The authors investigated the influence of platelet-rich plasma lysates on fibroblast proliferation as a function of pH in vitro. Fibroblasts were exposed to platelet lysates following preincubation at different pH values (5.0, 7.1, and 7.6), reporting that platelets preincubated at low pH (pH 5.0) induced the highest degree of fibroblast proliferation.

O'Toole et al.[43] reported findings associated with hypoxia and increased human keratinocyte motility. The authors utilized two independent migration assays to compare the motility of keratinocytes on connective tissue components under both hypoxic and normoxic conditions.

Results showed that human keratinocytes grown on a matrix of collagen or fibronectin exhibited increased motility when subjected to hypoxic (0.2 or 2% oxygen) conditions reflective of the wound environment (often modified by occlusive wound dressings) as compared with normoxic (9 or 20% oxygen) conditions. Furthermore, hypoxic keratinocytes showed increased lamellipodia-associated protein expression and distribution and decreased expression of laminin-5, which inhibits keratinocyte motility. Given that hypoxia is associated with a reduced pH, the study incorporated an experiment to determine the effect of pH as a factor independent of hypoxia. This investigation revealed that progressively lower pH conditions led to a decrease in keratinocyte migration on type 1 collagen, independent of oxygen tension. However, keratinocytes incubated in a buffered system (pH 7.5) under hypoxic conditions retained their elevated migration capacity. The authors concluded that, although hypoxia may not be beneficial for overall wound healing, reepithelialization caused by an increase in cellular motility could well be initiated by these environmental changes.

Although the independent effect of pH was not highlighted, similar early work by Horikoshi et al.[44] demonstrated that keratinocytes seeded under low oxygen tensions proliferate at a faster rate than those cells seeded at high oxygen tension. Indeed, cells grew best at 18% oxygen with a mean population doubling time of 2.8 days compared with 98% inhibition of growth at 52% oxygen reflected by a mean cell doubling time of 58 days. No growth occurred at an oxygen tension of 89%. However, growth was retarded at both 5 and 1% oxygen. The results revealed that

keratinocytes grow better when the cells were seeded under low oxygen tension, which provided optimum attachment to the culture surface, and then maintained at an ambient oxygen tension.

Acidification of the stratum corneum (SC) is also an important factor in the activity of β-glucocerebrosidase, an enzyme which plays a role in the membrane structural maturation in mammalian SC.[45,46] Mauro and colleagues[46] examined the effect of pH on barrier recovery following an acute injury. It was found that recovery, as assessed by changes in transepidermal water loss (TEWL), proceeded normally when acetone-treated skin was exposed to solutions buffered to an acidic pH. In contrast, the initiation of barrier recovery was slowed when treated skin was exposed to neutral or alkaline pH.

EFFECT OF pH ON THE IMMUNOLOGICAL RESPONSE

Effect of pH on macrophages

An early study completed by Mraz and colleagues[47] demonstrated that tissue acidosis is important for the recruitment of macrophages to the wound site. Crowther et al.[48] noted a reduction in vascular perfusion in tissues resulted in ischemia with a consequent reduction in local oxygen and glucose. In turn, cells use anaerobic metabolism, with increases in lactate production and a reduction in extracellular pH. These changes in the microenvironment stimulated cells to produce proangiogenic cytokines and enzymes in order to restore the local vascular supply.

Bidani et al.[49] suggested that a diminished extracellular pH may result in suppressed cytokine production and cytotoxic effects which impair the immune response to infection. Heming et al.[50] also investigated the role of an acid–base environment on the activity of macrophages. The group used alveolar macrophages to study the effect of extracellular pH on the production of tumor necrosis factor-α (TNF-α), as measured by mRNA levels. Cells were activated by lipopolysaccharide and cultured at increasing pH values (pH 5.5, 6.5, or 7.4). The results indicated a pretranscription, post-transcription, and post-translational effect on TNF-α secretion. Overall, at low pH, TNF-α secretion was decreased. An earlier study by Jensen and colleagues[51] showed that the production of factors by macrophages which stimulate angiogenesis and mitogenesis can be influenced by changes in environmental pH. It was found that those cells cultured at pH 6.2 failed to produce angiogenic factors. In contrast, similar changes in the cellular environment did not influence the production of macrophage mitogens.

Effect of pH on polymorphonuclearcytes (PMNs)

Interestingly, a large amount of research has been conducted on leukocyte activity in the acidic pH environment. The first documented study investigating the effect of pH on leukocytes was carried out by Nahas and colleagues in 1971[52] who investigated the rate of random leukocyte motility. When pH was decreased from 7.4 to 6.5, adverse effects such as a loss of leukocyte locomotion were showed. When pH was increased above 7.6, a significant decrease in locomotion was also reported. At pH 7.9, irreversible inhibition of motility was observed. Further to these findings, Rabinovitch and colleagues[53] found that an acidic pH inhibited chemotaxis of leukocytes, but at pH 6, random neutrophil movements were observed. Later, a study by Rotstein and colleagues[54] demonstrated impairment of chemotactic function when pH was reduced to pH 5.5. However, a more recent study by Leblebicioglu and colleagues[55] reported that chemotaxis in PMNs was impaired at pH 7.7 and 8.2 ($p < 0.05$). At a pH of 6.7, no affect on chemotaxis was found. Furthermore, phagocytosis of opsonized bacteria was decreased at pH 7.2 compared with pH 7.7. The authors concluded that collectively, these data suggest that environmental pH may selectively influence PMN activation and the balance between bacteria and host response. Further work by this research group confirmed that alkaline conditions increase apoptosis of PMNs.[56] PMNs were cultured under increasing pH conditions, and cells were stained for florescence microscopy for analysis of apoptosis over an 8-hour study period. After 3 hours of culture, 9% of PMNs cultured at pH 6.7 underwent apoptosis compared with 12% at pH 7.2, 38% at pH 7.7, and 60% at pH 8.2. It was also found, via the introduction of inhibitors to the culture medium, that serine proteases (SPs), caspase-1, and caspase-3 had a role in apoptosis caused by a rise in pH.[54] Thus, based on data currently available, chemotaxis and random migration of PMNs are impaired when extracellular pH becomes too acidic.

Craven and colleagues[57] investigated the influence of pH on neutrophil function by assessing phagocytosis of *Staphylococcus aureus*. Intracellular killing of *S. aureus* was not generally affected by extracellular acidification until pH was reduced to pH 5.0. Rotstein et al.[58] studied *Bacteroides* sp. and the effect of succinic acid on respiratory bursts in neutrophils. The researchers found that an inhibitory effect occurred at pH 5.5 but not at pH 7.4, suggesting succinic acid may induce an effect by reducing intracellular pH (pHi). Further study by this research group demonstrated how a reduction in the pH of the microenvironment of human neutrophils impairs migration.[54] A reduction in the pH value affected both random and chemotactic migration, as demonstrated in two experimental models: the agarose and Boyden chamber techniques. At a pH of 5.5, a significant reduction in PMN migration was noted ($p < 0.01$) as compared with a pH value of 7.5. Furthermore, a reduction in oxygen tension heightened this effect.[54]

Leblebicioglu and colleagues[55] found that the activation of the respiratory bursts in neutrophils was optimal at pH 7.2. They reported that these respiratory bursts decreased at pH 6.7 and 8.2. Furthermore, Lactoferrin release and phacocytosis of opsonized bacteria were shown to be inhibited at pH 7.2. Trevani and colleagues[59] found that neutrophil apoptosis was delayed at an acidic pH. Nakagawara et al.[60] reported that an increased rate of apoptosis in neutrophils occurred when the external pH increased. Similarly, Gabig et al. in 1979[61] revealed that at low oxygen tension and pH, representative of the physiological state of sites of infection, respiratory burst activity of neutrophils was reduced. Likewise, Allen et al.[62] found that the respiratory burst capacity of human blood and wound neutrophils, as measured by the production of superoxide and the consumption of oxygen, was impaired by a reduction in oxygen tension, representative of the wound milieu. To a lesser extent, it was also demonstrated that pH, temperature, and glucose concentra-

tion had a role to play in the regulation of neutrophil bacterial killing. Collectively, these findings suggest a role for these environmental factors in susceptibility of wounds to infection. It was shown by Gargan et al.[63] that neutrophil phagocytosis in urine was dependent on both osmolality and pH. Results revealed that phagocytosis by neutrophils was as good in urine as in Hanks balanced salt solution at both 485 and 200 mOsm at pH values between 6 and 8, whereas as at pH 5, phagocytosis of three bacterial strains was virtually abolished.

Coakley and colleagues[64] demonstrated how the physiologic environment of neutrophils can influence their activation and activity both in health and disease. Although neutrophils are a key component of the inflammatory process, there is a paradoxical implication for neutrophils in the pathogenesis of disease, where they are poorly regulated. The authors investigated the effects of altered pCO_2 on neutrophil activity and function. Given that alterations in carbon dioxide levels simultaneously affected pHi, the contribution of both of these factors in biological processes was investigated in terms of the cells' capacity for intracellular oxidant generation and interleukin (IL)-8 release. Alterations in pCO_2 of the culture medium caused changes in pHi and subsequent changes in oxidant production, such that neutrophils present at sites of low pCO_2 initiated or exaggerated the inflammatory response in comparison with intracellular acidification caused by high pCO_2, which may impair the inflammatory response. As elevation of pCO_2 and thus low pH values can occur in enclosed, perfused spaces particularly in the interstitial fluid in comparison with normal tissues, for example, in abscess cavities and tumors,[64–66] concurrent acidification of the neutrophil environment may affect healing, with chronic inflammation delaying healing progression. Acidification of the wound bed, therefore, may enhance healing by impounding the immune response. The extent to which this change in environment may aid healing remains unclear, and so, a clearer understanding of how direct and indirect immune function might be affected by changes in pH is required.[66]

Effect of pH on lymphocytes

Studies relating to the effects of external pH on lymphocytes are few and far between. One interesting study, however, by Ratner[67] investigated the effects of pH on the adherence and migration of lymphocytes through the extracellular matrix in an in vitro model, replicating the in vivo milieu. Murine splenic lymphocytes were activated by IL-2 then cultured in a three-dimensional gel composed of type I collagen. The effect of changing pH was monitored in terms of cellular motility through the three-dimensional matrix. In both gels, the results suggested that the acidification of the collagen matrix (pH 6.7 compared with pH 7.1) increased locomotory activity of motile lymphocytes, yet these changes did not overtly affect the recruitment of nonmotile cells. Interestingly, preincubation of these cells at pH 6.7 did not influence subsequent motility at pH 7.1. Although the author attempted to replicate the in vivo situation, collagen matrix models may not be truly indicative of the pathological environment where continuous fluctuations of a number of cellular mediators occurs, particularly in terms of acute and chronic wounds.

Effect of pH on complement activation and antibody production

In reference to complement activation, there are many publications that suggest that an acidic pH has a positive effect on its activation. Fishelson and colleagues[68] demonstrated that at pH 6.4, compared with 7.4, the alternative complement pathway increased lysis in sheep erythrocytes. It was concluded that a pH of 6.4 was optimal for both the initiation and amplification of the pathway and may also prove to be optimal for the activation of the membrane attack complex, a crucial part of the alternative pathway leading to cell lysis and death. In another early study by Hammer et al. in 1983,[69] it was shown that activation of C5 and C6 complement components is achieved in an acid environment, and that a high Histone H1 concentration causes a change in their tertiary structure resulting in the formation of a C5, 6a complex with subsequent cleavage and lytic capacity. Furthermore, Miyazawa and Inoue[70] found that C-reactive protein activates the complement activation system in slightly acidic conditions, even in the absence of their specific ligands. Raghavan and colleagues[71] demonstrated that immunoglobulin G binds to an Fc receptor, which aids in the delivery of the antibody to the bloodstream, more efficiently at pH 6–6.5 compared with pH 7.5. Furthermore, in a study completed by Udaykumar and Saxena,[72] acid-treated tuberculosis sera (as low as pH 2.8) were found to result in greater titers of antibodies to *Mycobacterium tuberculosis*, and acid-treated antibodies retained their specificity.[72]

Overall findings regarding the effect of pH on immunological functions

It is evident from the literature to date that pH has an effect on immunological responses in the human host, specifically on cell-mediated immunity. Many studies have found that both complement activation and antibody synthesis are enhanced at an acidic pH. However, cell-mediated immunity studies have, in general, only been undertaken in nonphysiological buffered in vitro models, therefore affecting clear findings relevant to the in vivo environment. Nevertheless, based on early work, it has been shown that an environment that is too acidic will result in a reduction in phagocytosis and diminished migration and chemotaxis of neutrophils. In conjunction with this, research by Trevani and colleagues[59] has suggested that an acidic pH can impair neutrophil function. Although it has been demonstrated in some studies that lymphocyte activity can be affected in an acidic microenvironment, only a small number of studies have been undertaken, particularly in models which mimic an in vivo wound environment.

Further studies are warranted in this area to assist with enhancing understanding of how pH may affect immunological function. It is interesting to consider that an acidic pH has an effect on neutrophils by suppressing their activity, particularly in terms of wound healing in chronic wounds which remain in a state of chronic inflammation and where neutrophil concentrations are generally high. Furthermore, the release of excessive amounts of elastase from these neutrophils can have detrimental effects on healing. Therefore, a more acidic pH would aid to dampen this excessive and destructive effect. It is important to note, however, that such an effect would be beneficial in chronic rather than acute wounds.

A review by Lardner in 2001[66] concluded that there was a shortage of data concerning the effects of pH on the immune response, in particular opsonization of bacteria, antibody synthesis and activation, hypersensitivity, and cytokine production. The author went on to suggest that the effect of environmental pH "will encourage more research in what is undoubtedly a field for future research."

EFFECT OF pH ON TISSUE ENZYME ACTIVITY

As emphasized by Schmid-Wendtner and Korting,[73] the skin displays a pH gradient across the SC which is likely to be important in the control of enzymatic activities and skin renewal. A number of studies (discussed below) have highlighted the effects of various contributing environmental factors, including pH, on the activities of enzymes and the resulting effects on tissue healing. It is also possible that changes in wound pH affect healing because of inhibition of endogenous and therapeutically applied enzymes given that the conformational structure and thus function are altered under the changing environmental conditions seen during the phases of healing.[74]

Matrix metalloproteinases (MMPs) and other enzymes

MMPs have an important role to play in remodeling the extracellular matrix for effective wound healing. However, when they are present in high, uncontrolled amounts, as evident in chronic wounds, their actions can have a detrimental effect on "normal" wound healing. The overabundance of enzymes in a chronic wound will lead to the degradation of many vital components necessary for the remodeling of the extracellular matrix.[10,75]

Enzymatic activity is known to be affected by various factors, in particular pH. For example, at a pH of 8, elastase, MMP-2, and plasmin activity is optimized, whereas neutrophil elastase has an optimum activity at pH 8.3.[2,31] However, hyaluronidase, a protease with high activity in chronic wounds as compared with acute wounds,[75] shows optimal activity at a low pH.[76,77] Similarly, SC thiol protease, an epidermal specific protease secreted by keratinocytes, has an acidic pH optimum.[78] The optimum pH range for the collagenase enzymes is pH 6–8, in contrast to fibrolysin which has a pH optimum of 7–8 and pH 4.5–5.5 for DNAase.[79] These variations in enzyme pH optima further highlight that various stages in the wound repair process require different pH milieus.

Serine proteases (SPs)

Hachem and colleagues[80] used TEWL analysis to investigate the effects of long-term neutralization of the SC on skin barrier function in relation to changes in SP activity. In a previous study, the authors found that deleterious effects occurred as a result of an elevated pH which was linked to high SP activity during short-term neutralization; SP inhibitors were shown to normalize SC integrity and cohesion even when pH was elevated.[81] In the later study, a sustained elevation in pH was applied without introducing confounding variables because of buffer use, inhibitor treatment, or knockout animal models. The results demonstrated that a sustained neutralization of the SC caused abnormalities in SC integrity and cohesion which were attributable to the sustained SP activity, which in turn accelerated corneodesmosome (the structures which hold the corneocytes together) degradation.

Lipid processing enzymes

Hachem et al.[81] went on to investigate the role of β-glucocerebrosidase and acidic sphingomyyelinase (lipid-processing enzymes with known acidic pH optima) in skin barrier function using in situ zymography. It was found that during sustained neutralization of the skin, both of these enzymes showed reduced activity, and their activity did not return to normal levels following the reintroduction of an acidic pH environment. The degradation of these enzymes was suggested as a mechanism for abnormal lipid processing and thus barrier abnormalities during sustained periods of raised pH.[81]

Bacterial proteases

As well as human enzymes, microbial proteases are known to have an effect on wound healing and are highly prevalent in chronic wounds. The activity of bacterial enzymes can often lead to an increase in the alkalinity of the wound and skin environment increasing the wounds propensity to infection.[10] *Proteus mirabilis*, *Klebsiella* sp., and *P. aeruginosa* are known to produce the enzyme urease.[10] Urease is important to bacteria as it is used to liberate ammonia from urea to reduce the detrimental effects of an acidic environment found on the skin surface, and therefore, it aids in microbial attachment and proliferation.[14,21] Consequently, a change in a wound's pH will help to enhance the conditions of the bacteria's microenvironment which will aid growth, therefore increasing the wounds microbial bioburden and heightening the risk of infection[9,14] resulting in a positive feedback mechanism causing yet more bacterial enzymes to be produced.

An enhancement of bacterial protease activity has also been indicated in the heightened activity of host enzymes. For example, Twining et al.[82] sought to study the effects of proteases released from *P. aeruginosa* on corneal proteinase activity and found that *P. aeruginosa* elastase cleaved host corneal pro-MMPs into their active forms, and both elastase and alkaline protease derived from this pathogen acted in the degradation of caseinase. Similarly, a stimulatory effect on collagen degradation by proteases secreted by keratinocytes has been, in part, attributed to the excess of *P. aeruginosa* elastase. This bacterial enzyme was capable of directly degrading type I collagen in an in vitro collagen matrix model, and elastase was also able to activate host pro-MMPs (MMP-1, -2, -3, and -9).[83] Other bacterial proteinases important in the augmentation of tissue degradation include LasA protease and protease IV derived from *P. aeruginosa* and cysteine protease and metalloproteases produced by *Serratia marcescens*. These bacterial enzymes can affect the degradation of tissue constituents and host-defense proteins alongside the activation of zymogens.[84]

Enzymatic activity and pH can have an effect on oxygen availability, a factor considered significant to wound healing.[85] For example, a lowering of the pH by just 0.6 units is reported to enhance the release of almost 50% more oxygen into the

wound to aid healing.[21,86] Therefore, in a chronic wound, the likelihood of healing is high if tissue oxygen tension is >40 mmHg, but it is unlikely at levels <20 mmHg.[2,87]

EFFECT OF pH ON MICROBIAL VIRULENCE AND PATHOGENICITY

Although it is difficult to investigate the environmental factors involved in the pathogenesis of disease relating to bacterial gene expression, a number of studies have identified possible factors which may lead to changes in bacterial virulence, including the local pH. In 2004, Weinrick and colleagues[88] sought to examine the effects of nutrient supply and other chemicals on the expression of virulence genes during the growth of *S. aureus* in vitro. Through transcriptome profiling via microarray analysis, the authors found that under acidic conditions (pH 5.5 vs. pH 7.5), the transcript level of *S. aureus* genes differed by at least twofold. Rippke et al.[89] also noted how bacterial growth and virulence of *S. aureus* alongside defensive host mechanisms may, in part, be influenced by the changes in the skin's pH in atopic dermatitis. Harjai and colleagues[90] studied the effects of pH on the virulence of *P. aeruginosa*, a common wound pathogen, in the biofilm mode of growth. The researchers found that at an elevated pH value of 8, alginate and proteinase production was increased, whereas at lower pH (pH 5), an increase in the production of siderophores (high-affinity iron-chelating compounds) was noted. The attachment rate of flagellated bacteria is also greatly dependent on temperature and pH as shown by the attachment of various bacterial strains to the skin of broiler chickens.[91]

It has been shown that an acidic environment can stimulate group A *Streptococcus* (a Gram-positive bacteria implicated in many human diseases including skin infections) to express streptococcal pyrogenic exotoxin B (SpeB).[92] SpeB is a cysteine protease that has important roles in group A *Streptococcus* pathogenesis. The exotoxin is regulated by the autoinducer-2/LuxS signaling pathway which participates in quorum sensing. The caseinolytic activity of SpeB, however, was shown to be optimal at pH 8.[93]

As pathogenic fungi can adapt to changing environments and attack a range of hosts, they display ambient adaptation including adaptation to changes in pH.[94] Prusky and Yakoby[94] described the pH sensing-response system which allows fungi to adapt its virulence to suit its changing environment. These pathogens are also able to actively increase or decrease their environmental pH in order to adapt virulence to suit a particular host.[95]

BIOFILMS AND THE EFFECT OF pH

Biofilm formation is a key virulence factor in the survival of pathogens in diverse milieus. The biofilm represents a protected mode of growth for these microorganisms, allowing cells to survive in hostile environments and disperse themselves in order to form new colonies in other areas of the host environment.[95] Although many bacteria have a narrow pH range for growth, when they are present in biofilm communities, they display the ability to survive within a pH range that would normally be inhibitory to their growth under planktonic conditions.[96] Biofilms consist of microorganisms which are entrenched within a matrix of extracellular polymeric substances and attached to each other, or to a nonbiological or biological surface/support. Phenotypically, the microorganisms in this state differ significantly from their planktonic counterparts. This has included characteristics such as growth rate, gene transcription, antimicrobial sensitivity, and enzymatic activity.[97] The effect of pH (5.5, 7.5, 8.5) on biofilm production has been compared in *P. aeruginosa*, *Klebsiella pneumoniae*, and *Vibrio cholerae* non-O1 and O1 using a crystal violet test.[12] The researchers found that an increased pH led to a higher biofilm production. *P. aeruginosa* biofilm production was reported at 139–244% and 136–164% higher at pH levels of 8.5 and 7.5, respectively, when compared with biofilm production at pH 5.5. For *K. pneumoniae*, biofilm production increased to 151–319% at pH 8.5, whereas at pH 7.5, the biofilm production was 113–177% higher when compared with pH 5.5. For *V. cholerae*, non-O1 and O1 the biofilm production reached 204–329% higher at pH 8.5, and 123–316% higher at pH 7.5 when compared with production at pH 5.5. The increased biofilm capacity represented an average of 169% when pH was increased from 5.5 to 7.5, whereas the rise in pH from 5.5 to 8.5 caused an average difference of 229%.

Studies which have been carried out on the effects of pH changes on shifts in bacterial load in the biofilm state have revealed that under these changing environments, the growth rate of different bacteria changes within the local microbial community. One of the main areas of focus in terms of these biofilm growth changes in mixed cultures is within oral microbial communities. Although such studies may also be reflective of the situation in wound tissues, more research in this area is needed to make more conclusive judgments. Bradshaw et al.[98] investigated the influence of both glucose availability and pH in combination (given that a reduction in pH is consequential of glucose metabolism) or as independent factors on the proportions of various bacteria within the biofilm community. Using chemostats for biofilm growth under standardized conditions, the authors noted that when glucose was administered under a stabilized neutral pH, little effect was seen in terms of microflora composition. In contrast, in the biofilm mode of growth where the pH was allowed to decrease following glucose administration, a remarkable difference was observed in the microflora composition, with some organisms increasing in both absolute numbers and as a proportion of the total count of the microbial community and others decreasing. A later study by Bradshaw and Marsh[99] confirmed their previous findings, with the decrease in some of the bacterial counts being linked to the magnitude of the pH fall. Li et al.[100] examined the acid tolerance response (ATR) of *S. mutans* in the biofilm mode of growth in order to determine the effect of cell density on the induction of adaptation to an acid environment and to differentiate between biofilm phenotype and cell density in terms of acid tolerance. Overall, they found that cell density and the biofilm state affected the adaptation of this bacterium to a decrease in pH, with cells at a higher density or those grown in a dense biofilm showing significantly greater acid resistance in the chemostat-based biofilm fermenter and broth culture models. Interestingly, a more recent study by Welin et al. in 2003[101] demonstrated that although planktonic *S. mutans* cells induced a strong ATR at low pH, biofilm cells displayed a stronger inherent acid resistance despite a minimal induction of an ATR system, as demonstrated by protein analysis using pulse labeling with [^{14}C]-amino acids and two-dimensional gel electrophoresis

followed by autoradiography and computer analysis, following a change in culture condition from pH 7.5 to 5.5.

Similarly, nine oral bacteria were grown in a glucose-limited chemostat to evaluate the effect of lowered pH on bacterial composition and metabolism in the biofilm community, in a study conducted by McDermid and colleagues.[102] The group found that a decrease in pH (pH 4.1) led to increased bacterial aggregation and altered flora composition and metabolism. Specifically, *Streptococcus mitior*, *Veillonella alcalescens*, and *Streptococcus sanguis* were the predominant bacteria at pH 7.0, whereas at pH 4.1, all bacterial counts were decreased apart from *Lactobacillus casei* and *S. mutans*. *Bacteroides intermedius* was not recovered below a pH value of 4.6. However, when normal pH levels were restored, the majority of bacterial species returned to normal in terms of growth and metabolism.

In a study by Stoodley et al.,[103] mixed species of bacteria were grown, including *Klebsiella pneumoniae*, *Pseudomonas fluorescens*, and *P. aeruginosa* in a flow cell fitted with wire electrodes to study the influence of electric fields and pH on biofilm structure. Following an initial growth phase in this model, a voltage was applied which caused the biofilm to expand and contract. This effect was also seen when media adjusted to either pH 3 or 10 was alternately introduced to the biofilm model without the electric current. Although the pH 10 media had no effect on biofilm formation, the biofilm contracted to 69% of its original depth upon administration of the pH 3 media. The authors concluded that the changes seen in biofilm conformation under acidic conditions were reflective of the molecular interactions between charged acidic groups in the biofilm slime and bacterial cell walls. The nature of these effects, however, requires further investigation in terms of the true in vivo environment.

EFFECT OF pH CHANGES ON QUORUM SENSING

Horswill and colleagues[104] reviewed the effects of the microbial environment on quorum sensing in the biofilm mode of growth, highlighting the imperative assertion that optimizing experimental models to mimic the natural or clinical environment could be essential in terms of identifying when and where quorum sensing occurs in vivo. The authors noted an important critique of traditional quorum-sensing models in terms of producing reliable data representative of the "true" in vivo milieu; liquid-batch cultures represent a closed system which does not parallel the open, flowing system of the in vivo environment where the concentration of signal molecules varies depending on a number of factors.

As quorum-sensing systems affect bacterial virulence, biofilm formation, and antibiotic sensitivity,[105] it is therefore reasonable to allude that alterations in pH, whether these may be natural environmental changes or deliberate therapeutic changes, can have a considerable indirect effect on various aspects of bacterial virulence. One example of how quorum sensing affects virulence is the las and rhl quorum-sensing systems in *P. aeruginosa* which regulate virulence gene expression, including elastase and rhamnolipid production.[106] Furthermore, the quorum-sensing molecule 3O-C12-HSL is a potent stimulator of a number of eukaryotic cells, indicating that host response to infection by *P. aeruginosa* may be affected by the stimulation of quorum-sensing systems.[107] Indeed, in the absence of one or more quorum-sensing components, virulence is hampered.[107] Microarray analysis has shown that environmental factors including oxygen tension and medium composition affect quorum sensing in *P. aeruginosa*, with the elimination of transcripts of a number of genes regulated by quorum sensing under varying environmental conditions.[105]

EFFECT OF pH ON THE PERFORMANCE OF ANTIBIOTICS

Antibiotics are employed in medicine for killing harmful pathogens at local or systemic levels. They are administered at therapeutic dosages for treating bacterial and fungal infections in wounds. Worldwide, there is an overuse and misuse of antibiotics which has led to increasing failures of antibiotic therapies because of emerging increased resistance to antibiotics in many strains of microorganisms, inappropriate prescribing, and poor regulation in many countries.[108–111]

All antibiotic susceptibility testings of bacteria isolated from wounds are assessed by the disk diffusion assay utilizing commercially available antibiotic disks. However, there are recognized limitations of the zone of inhibition (ZOI) test for accurately evaluating the antibiotic sensitivity of bacteria, particularly when bacteria are residing in the biofilm phenotypic state.[112] Clutterbuck and colleagues[112] employed the use of a biofilm poloxamer ZOI assay to compare the antibiotic sensitivity testing of bacteria both in the planktonic and biofilm phenotypic states. Poloxamer is a di-block copolymer of polyoxyethylene and polyoxypropylene and has been reported as an agent that can be used to grow biofilms and study the antimicrobial performance of biocides.[112–118]

pH has been known to affect the performance of antibiotics.[119] It is probable that pH affects antibiotic efficacy by modulating the binding and/or target sites for certain antibiotics and possibly not by making bacteria more or less tolerant to antibiotics. It has been found that antibiotics such as gentamicin are prevented from being transported into bacteria specifically in an acidic environment.[120] The hypothesis proposed was that this impairment was because of a larger ionization of the antibiotic at a more acidic pH compared with a neutral pH. However, increased tolerance to antimicrobials at certain pH ranges may be because of alterations of the metabolic state of bacteria, in particular the generation of small colony variants.[121] Small colony variants are known to be intrinsically more tolerant to antibiotics.[122]

Lamp and colleagues[123] found that as pH increases, the activity of antibiotic activity against *S. aureus* also increases. Furthermore, the fluoroquinolones, in particular finafloxacin, has been shown to have enhanced activity under slightly acidic conditions (pH 5.0–6.5).[124] Other antibiotics that include streptomycin and erythromycin have also been reported to be affected by pH.[125,126]

The minimal inhibitory concentrations (MIC) of certain bacteria to antibiotics have been found to be lower at high pH compared with neutral or low pH. Such a pH effect on antibiotic performance has been demonstrated in *Staphylococci*.[127] For erythromycin, however, it has been found that at alkaline pH Gram-negative bacteria are more susceptible.[128] The increased efficacy of erythromycin in alkaline conditions has also been reported by Lorian et al.[125] when compared with efficacy in a neutral or acid environment. Baudoux and col-

leagues[129] evaluated the efficacy of gentamicin and oxacillin and the effects of pH toward *S. aureus*. The researchers found that MICs and minimum bactericidal concentrations increased 72-fold for gentamicin and decreased eightfold for oxacillin between pH 7.4 and 5.0. pH has also been shown to have an effect on macrolides and aminoglycosides.[130] Furthermore, studies have also reported that lowering pH can lead to higher MICs for other ranges of antibiotics.[131,132] It has been found that both macrolides and quinolones, particularly ciprofloxacin, demonstrate a loss of activity at acid pH.[133] Consideration of the effect of pH on drug efficacy would therefore seem warranted when choosing an antibiotic for clinical use. An acidic environment has been shown to cause a decrease in MICs of beta-lactams when compared with activity at a neutral pH.[134]

EFFECT OF pH ON THE PERFORMANCE OF ANTISEPTICS

The effect pH may have on antiseptic efficacy, including popular wound antiseptics such as iodine and silver, has been poorly documented. This is despite some early research reporting that pH is known to affect the chemical speciation and bioavailability of metal ions.[135] The bioavailability of active free metal ions specifically in a wound will be affected by numerous factors including cationic exchange, ability to form complexes, precipitation, and adsorption.[136] All these factors are affected by pH with metal ion solubility known to increase when pH decreases.[137]

As mentioned previously, a nonhealing wound generally resides in a slightly alkaline environment. This will affect the availability of free, reactive, and active metal ions, including ionic silver at this pH as metal ions will be limited in solution by precipitation at alkaline pH values.[138] Collins and Stotzky[138] have shown that many metal ions precipitate between pH 3.0 and 6.0, and at a pH above 6.0, the microbial toxicity of a metal ion will be reduced.

Other antiseptics such as chlorhexidine and quaternary ammonium compounds have been found to be more active at an alkaline pH.[139] Contrary to this, antimicrobials such as hypochlorites are more active at an acid pH.[139]

As the pH of a wound is reported to have a role to play in affecting wound healing,[140] it is important to determine whether pH has an effect on the antimicrobial efficacy of antimicrobials, particularly ionic silver. The availability of ionic silver can be severely reduced in a chronic wound, particularly in those that contain large amounts of ions such as Cl^-, HCO_3^-, CO_3^-, as well as those containing high amounts of proteins and polysaccharides.[141] As silver ions interact with many ions within the wound, the production of insoluble silver salts will be high. This will lead to a reduction in the availability of ionic silver to kill microorganisms. The bioavailability of active ionic silver in a wound dressing needs to be high and sustained for long enough to maintain an effective microbial kill. However, as different pH ranges exist within a wound, this will affect biochemical stability and bioavailability of ionic compounds.[142]

EFFECTIVE OF pH ON OTHER ANTIMICROBIALS

Lee and colleagues[143] reported that antimicrobial peptides were more effective at a pH of 5.5 than at a pH of 7.5.[143] In addition to this, Minahk and colleagues have reported that the activity of enterocin CRL35 is higher at an acidic pH compared with that of a neutral or basic environment.[144] Honey has also been shown to have a positive effect on wound healing by effectively lowering pH.[15]

CONCLUSION

Chronic wounds have frequently been shown to contain elevated levels of both host and bacterial-derived proteases which can delay the healing process. These proteases, including MMPs and neutrophil elastase, augment tissue and cytokine destruction. Products which lower the levels of these proteases in the wound environment help to prevent the breakdown of extracellular matrix proteins and growth factors that are crucial for wound repair.[145] In a study by Edwards and Howley in 2007,[145] phosphorylated cotton dressings were tested for their ability to sequester elastase and collagenase activity in vitro. Results showed that under an acidic environment, alongside phosphorylation because of protonated phosphate at the dressing surface led to an increase in the efficiency of the dressing in terms of protease activity depletion.

Thorough investigation into the role pH plays in wound healing is urgently required. A number of wound-related processes have been shown to be affected in some way to changes in pH, and changes in pH may have either positive or negative effects on wound healing. Thomas[14] concluded that it would appear that dressings that directly or indirectly reduce the pH of wound fluid may help to prevent infection and will be likely to produce conditions that are more conducive to rapid healing than other materials which produce a more alkaline local environment. Furthermore, Greener and colleagues[31] suggested that proteolytic activity at the wound bed is sensitive to changes in pH, and modulating the pH to a more acidic environment may be another useful intervention for nonhealing wounds. However, eliminating all proteolytic activity from the wound is undesirable as this is essential for effective wound healing, but rebalancing the MMP/tissue inhibitors of metalloproteinase relationship would help reestablish the normal healing cascade. Leveen et al.[21] made the comment that oxyhemoglobin releases its oxygen more readily in an acid environment. As pH decreases, the standard oxyhemoglobin dissociation curve is moved to the right. A shift of the pH by 0.6 (down) almost doubles the quantity of oxygen released.

The pH value within the wound has been shown to influence indirectly and directly all biochemical processes which are important for healing. In particular, pH has been shown to have a critical role to play in the healing of wounds as pH is known to have an effect on underlying pathophysiology, biochemistry, immunology, and microbiology. Historically, it has been assumed that a low pH value, such as is found on normal skin, is more favorable for wound healing. pH also has been shown to have a role to play in determining antimicrobial activity and performance and therefore the effective treatment when a wound infection occurs. However, pH has only been relatively recently considered as an explanation as to why many treatment approaches fail to eradicate infections in chronic wounds.[126] In general, lowering pH has shown to result in an improvement in the antimicrobial activity of some antimicrobials, in particular those that contain silver.[146] It is therefore possible to suggest that there may be benefits to maintaining or "pushing" an infected wound to a slightly acid

environment. This could potentially lead to an enhanced antimicrobial effect of silver, a quicker reduction in the wound microbial bioburden, and therefore a reduction in the need for prolonged antimicrobial usage. However, further in vitro and in vivo studies would be warranted to further substantiate these claims.

This review paper has summarized the existing literature which has dealt with pH within the wound and the role it plays in healing, biofilm formation, and antimicrobial performance for effective management of wounds. It is clear that pH has a role to play in wound healing, influencing the phases of wound healing, microbial proliferation, enzymatic activity, immunology, biofilm virulence (including biofilm formation), and performance of treatment regimes presently employed in wound care. A lot of further work on pH in both healing and nonhealing wounds is required so that future technologies can be developed that are pH specific with the aim to achieve quicker and effective wound healing.

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