REVIEW ARTICLE

The Effect of pH on the Antimicrobial Efficiency of Silver Alginate on Chronic Wound Isolates

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Abstract

BACKGROUND: Nonhealing and stalled chronic wounds are often reported to reside within an alkaline environment. Consequently, a number of researchers have proposed that lowering the pH of a chronic wound environment will enable healing to progress. However, it is not known whether the efficacies of silver-impregnated wound dressings are affected by pH.

OBJECTIVE: To investigate whether pH has an effect on the antimicrobial barrier efficacy of a silver alginate wound dressing on wound isolates.

METHODS: Twenty-five bacteria and yeasts that had been routinely isolated from chronic wounds were separately exposed to a silver alginate wound dressing with the use of a standardized corrected zone of inhibition (CZOI) assay.

RESULTS: The silver alginate dressing demonstrated a broad spectrum of antimicrobial barrier activity within the dressing against all wound isolates. However, at a pH of 5.5, compared with a pH of 7, the antimicrobial barrier activity of the silver alginate dressing significantly increased. For all yeasts the CZOI ranged from 6.25 to 11 mm at a pH of 7. At a pH of 5.5, the CZOI range increased from 8.5 to 12.25 mm. For the Gram-negative isolates, the CZOI ranged from 0.75 to 6.5 mm at a pH 7, compared with a CZOI range of 2.75 to 8 mm at pH 5.5. The CZOI for the Gram-positive isolates, including methicillin-resistant *Staphylococcus aureus*, ranged from 3 to 7.75 mm at pH 7 and from 4.5 to 11.75 mm at pH 5.5.

CONCLUSION: For all isolates tested, excluding one strain of *Candida albicans* and one vancomycin-resistant *Enterococcus* strain, lowering pH to 5.5 resulted in an improvement in the antimicrobial barrier activity within the silver alginate dressing. Based on these initial in vitro findings, it is possible to suggest that there may be benefits to maintaining an infected or recalcitrant wound in a slightly acid (pH 5.5) environment. In particular, doing so may lead to an enhanced antimicrobial barrier effect of silver, a quicker reduction in the wound microbial bioburden, and therefore a reduction in the need for prolonged antimicrobial use. However, more in vitro and in vivo studies would be warranted to further substantiate these claims.

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Introduction

During “normal” wound healing, a series of overlapping biological events occur, leading to effective healing. These biological events include hemostasis, inflammation, proliferation, and remodeling. In chronic wounds these biological

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events become unsystematic and uncontrollable, leading to a “stalled” nonhealing or infected state. The pH of a wound has been shown to affect many of the biological processes necessary for effective healing, including promoting angiogenesis, increasing fibroblast activity, and increasing macrophage activity.¹ Changes in pH are also known to have an effect on the activity of enzymes, particularly human matrix metalloproteinases (MMPs).^{1–4} For example, enzymes such as elastase, MMP-2, and plasmin have optimum activity at a pH of 8, compared with neutrophil elastase at 8.3.^{1,5} Although human MMPs have an important role to play in wound healing, when they are produced in large amounts and are uncontrolled, their actions become detrimental to wound healing. The reason for this behavior is that excessive unregulated enzymatic activity leads to the degradation of many components in the extracellular matrix.⁴

Often overlooked in wound care is the importance of proteases and other enzymes produced by bacteria and fungi.⁴ The abundance of microbial enzymes in a wound is significant to healing rates, with their activity often leading to an increase in the alkalinity of the wound environment.⁴ For example, the urease enzyme produced by many bacteria, including *Proteus mirabilis*, *Klebsiella* spp, and *Pseudomonas aeruginosa*, in a wound liberates ammonia from urea.^{6–9} Consequently, high urease activity in a wound leads to an increase in ammonia concentrations and therefore a more alkaline environment. This change in pH will lead to a deterioration of the wound and a heightened risk of infection.^{3,7}

Modulation of enzymes is an integral part of wound bed preparation, and presently used methods of achieving modulation include debridement and wound irrigation with topical antimicrobials.¹⁰ Silver in particular has been shown to be very effective in suppressing the activity of MMPs.¹¹ An additional method of MMP control could involve the adjustment of the wound pH.^{5,12} In addition to affecting enzymatic activity, pH influences oxygen availability. For example, a lowering of pH by just 0.6 units is reported to enhance the release of almost 50% more oxygen into a wound.⁶ In a chronic wound, the likelihood of healing is high if tissue oxygen tension is >40 mm Hg but unlikely at levels <20 mm Hg.¹³

It has been suggested that pH may be a good predictor of wound healing.^{2,6,14,15} For example, Roberts and colleagues¹⁴ demonstrated that wounds with an elevated alkaline pH had a lower healing rate than did wounds with a pH closer to neutral. Hoffman and colleagues¹⁶ also reported that in alkaline conditions, wound healing does not progress well, and Gethin¹ reported that both acute and chronic wounds move from an alkaline state to a neutral and then to an acidic state when the wound begins to progress to healing. Consequently, as pH is purported to have a significant role to play in wound healing, we wanted to investigate the effect that pH may have on the antimicrobial efficacy of a silver-containing wound dressing. Many metals such as silver, in their ionic forms, demonstrate

very good antimicrobial qualities. However, pH is known to affect the chemical speciation and bioavailability of metal ions.¹⁷ The bioavailability of active free metal ions in a wound is dependent on a range of factors, including cationic exchange, ability to form complexes, precipitation, and adsorption. All these conditions are influenced by pH.¹⁸ However, most metal ion solubility increases when pH decreases.¹⁹ Consequently, the availability of free, reactive, and active ionic silver may be limited in solution by precipitation at higher alkaline pH values. In fact, many metal ions precipitate between pH 3.0 and 6.0.²⁰ Therefore, at a pH above 6.0, there would be a reduction in microbial toxicity of metal ions. We decided to investigate, using a rapid corrected zone of inhibition (CZOI) screening assay, whether pH had an effect on the antimicrobial barrier efficacy of a silver alginate dressing.

Methods

Test Microorganism

Twenty-five clinical isolates that had been routinely isolated from chronic wound patients at the West Virginia University Hospital, West Virginia, USA, were used in this study. The diversity of microorganisms evaluated is included in the Table. All purified bacteria and yeasts were allowed to grow at 37 °C for 18 to 24 hours on blood agar plates and then inoculated into 10 mL of tryptic soy broth and vortexed before being used in all tests.

Test Dressings and Materials

The silver-containing wound dressing that was evaluated in this study was a silver alginate dressing (AMS, Winsford, UK). The control was a nonantimicrobial gauze dressing.

Each test organism was inoculated into a 70-μL sample of tryptic soy broth and then added to 5 mL of blood bank saline (0.85%). Inoculated saline (containing 1×10^6 colony-forming units per milliliter) was then swabbed (with a sterile cotton swab) onto Mueller Hinton Agar (MHA) plates, according to Clinical Laboratory Standards Institute techniques. MHA plates were divided into segments to incorporate 1-cm² dressings. All wound dressings (1 cm²) were presoaked in saline for 30 seconds, drip dried, and placed strategically onto an inoculated MHA plate. All plates were then incubated at 37 °C for 24 hours. After incubation the CZOIs were recorded for the wound dressings. The CZOI was measured vertically and horizontally in millimeters for the test and control wound dressings. To demonstrate the effects of pH on silver efficacy, MHA plates were adjusted to a pH of 5.5 or 7 (by the addition of hydrochloric acid or sodium hydroxide during media preparation). All experiments were done in triplicate.

Table The Diversity of Chronic Wound Microorganisms Evaluated

Organism	Gram	Number Evaluated
<i>Candida albicans</i>	NA	3
Yeast	NA	2
<i>Pseudomonas aeruginosa</i>	negative	2
<i>Acinetobacter baumannii</i>	negative	1
<i>Citrobacter freundii</i>	negative	1
<i>Escherichia coli</i>	negative	1
<i>Salmonella</i> spp	negative	1
Meticillin-resistant <i>Staphylococcus aureus</i>	positive	5
<i>Staphylococcus aureus</i>	positive	2
Vancomycin-resistant enterococci	positive	5
<i>Enterococcus faecium</i>	positive	1
<i>Staphylococcus lugdunensis</i>	positive	1

Statistical Analysis

A Student *t* test was used to compare CZOI between pH ranges. All data were analyzed with the use of Microsoft Excel.

Results

The silver alginate dressing demonstrated a broad spectrum of activity against all 25 clinical isolates studied at both pH 7 and pH 5.5. However, it was found that pH had a significant effect on the antimicrobial barrier efficacy of the silver alginate dressing. For example, the CZOI for the silver alginate dressing against *Candida albicans* and yeast ranged from 6.25 mm to 11 mm at pH 7, compared with a CZOI range of 8.5 mm to 12.25 mm at pH 5.5 (Figure 1). A similar effect was seen for all gram-negative bacteria evaluated. For *Pseudomonas aeruginosa*, for example, the

CZOI at a pH 7 was 5.5 mm, compared with 7.5 mm at pH 5.5 (Figure 2).

For the Gram-positive bacteria, pH was also found to have an effect on the antimicrobial barrier activity of the silver alginate dressing. A change in pH from 7 to 5.5 resulted in a significant improvement on the activity of silver on MRSA and vancomycin-resistant Enterococci in particular (Figure 3; $P < 0.05$). It is interesting that at a pH of 5.5, all but 2 strains of microorganisms were found to be more sensitive to the efficacy of ionic silver.

Discussion

Advanced wound dressings are important to wound care for those wounds that are exuding moderate to heavy levels of wound fluid. Silver alginate wound dressings have good absorbency characteristics and, being composed of alginic acid, demonstrate good absorptive qualities over a wide pH range. However, the absorptive capacity of other wound dressings, such as those containing 100% carboxymethyl cellulose, can be affected by pH. In particular, an acidic pH has been shown to dramatically reduce the absorptive capacity of carboxymethyl cellulose fibers. Such an effect would be a concern to wound healing, particularly as chronic wound fluid is known to exist within a broad pH range, with a slightly acidic pH being common. A wound dressing that has the ability to reduce wound pH, combined with increased antimicrobial performance over a wide pH range, will be of great benefit to wound healing.

While the pH of a chronic wound has been reported to be in the range of 7.15 to 8.9,^{2,21,22} healing is a complex process known to affect, and be affected by, changes in pH.^{2,6,15,23} In chronic wounds that are at risk of becoming infected or are actually infected, an antimicrobial wound dressing such as a silver alginate is warranted.^{24,25} Consequently, as the pH of a wound is reported to have a role to play in wound healing,²⁶ it is important to determine

Figure 1 The Effect of pH on the Efficacy of Silver Alginate on *Candida albicans* and Yeasts CZOI, corrected zone of inhibition.

Figure 2 The Effect of pH on the Efficacy of Silver Alginate on Gram-Negative Bacteria CZOI, corrected zone of inhibition.

whether pH has an effect on the antimicrobial barrier 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^- , and CO_3^- , as well as those containing high amounts of proteins and polysaccharides.²⁷ 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 to be sustained long enough, to maintain an effective microbial kill. However, different pH ranges exist within a wound, which will affect biochemical stability and bioavailability of ionic compounds. Consequently, our aim was to evaluate the antimicrobial efficacy of a silver alginate

dressing at different pH levels and to determine whether a silver alginate wound dressing remained effectual.

Our study demonstrated that the silver alginate dressing was very effective in killing a broad range of commonly encountered wound microorganisms at pH of both 5.5 and 7. It is interesting that at a pH of 5.5, the antimicrobial efficacy of the silver alginate dressing was significantly enhanced, compared with its efficacy at pH of 7.0. This pH effect was demonstrated on Gram-positive and -negative bacteria, as well as yeasts. On the basis of these initial in vitro findings, we conclude that there may be benefits in helping to decrease the infected chronic wound pH to a slightly acidic (pH 5.5) state.

Aside from silver, however, the chassis itself has a role to play in wound healing. In this study alginates were used. Alginates are naturally acidic; consequently, alginates have

Figure 3 The Effect of pH on the Efficacy of Silver Alginate on Gram-Positive Bacteria CZOI, corrected zone of inhibition; MRSA, methicillin-resistant *Staphylococcus aureus*; VRE, vancomycin-resistant enterococci.

an inherent ability to produce slightly acidic conditions in different environments, including the wound environment.²⁸ It is plausible to suggest that during wound management, the use of silver alginate dressings may help in decreasing the pH of the wound environment. Such a reduction in pH will help to increase the antimicrobial barrier activity of silver and also assist in enhancing the progression of a wound to healing.²⁹ Such a hypothesis seems feasible to suggest, particularly as this theory has been conceptually demonstrated for other wound dressings.³⁰

Based on the findings of this study, and in conjunction with other research findings,^{11,30} it can be hypothesized that at an acidic pH, there is a significant reduction in MMP activity, a reduction in metal ion precipitation, and therefore an increase in the availability of ionic silver, leading to an enhanced antimicrobial barrier activity of silver. Consequently, within a more acidic wound environment, the increased availability of ionic silver will lead to a reduction of the wound microbial bioburden, a reduction in enzymatic activity, an increase in wound healing, and a reduction in the need for prolonged and unnecessary antimicrobial use.

In conclusion, lowering pH to 5.5 resulted in an improvement in the antimicrobial barrier activity of the silver alginate dressing. Based on these initial in vitro findings, it is possible to suggest that there may be benefits to maintaining an infected or recalcitrant wound in a slightly acid (pH 5.5) environment. Doing 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 use. However, additional in vitro and in vivo studies would be warranted to further substantiate these claims.

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