pH Influence on Antibacterial Efficacy of Common Antiseptic Substances

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Key Words

Antiseptic · Chronic wound · pH · *Pseudomonas aeruginosa* · *Staphylococcus aureus*

Abstract

Background: Wound infection plays an important role in compromised wound healing. A high bioburden impairs healing and leads to formation of a chronic wound. Distinctly higher pH values were observed in chronic wounds compared to acute wounds. However, there is only limited knowledge of pH dependency on the antibacterial efficacy of common antimicrobial substances. **Methods:** This study investigated the pH influence on the antimicrobial efficacy of povidone (PVP)-iodine, silver nitrate, chlorhexidine, octenidine and polihexanide against *Staphylococcus aureus* and *Pseudomonas aeruginosa* using the agar diffusion test and microplate laser nephelometry. **Results:** The bactericidal activity of chlorhexidine and octenidine was mainly pH-independent in a pH range of 5.0–9.0. In contrast, polihexanide showed a significant efficacy increase at a higher pH. It was also found that the influence of the pH on antiseptics differs among species of bacteria. For instance, *S. aureus* exhibited an increasing sensitivity against silver nitrate with rising pH whereas the effect on *P. aeruginosa* was found to be distinctly decreased. The antimicrobial effect of PVP-iodine was strongly diminished with rising pH. **Conclusions:** The shift towards higher pH values in chronic wounds com-

pared to acute wounds makes it imperative to know whether the antimicrobial efficacy of applied antimicrobial substances is altered by different pH levels. The results suggest that application of polihexanide might be advantageous for the management of wound infections, as both *S. aureus* and *P. aeruginosa* exhibited an increased susceptibility with rising pH.

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Introduction

An increasing number of patients suffer from wounds that fail to heal. This impaired wound healing derives from an imbalance between degradation and remodeling [1, 2]. Several studies have shown that exudates from nonhealing wounds contain elevated levels of proteases, like matrix metalloproteinases and polymorphonuclear elastase [3–5]. In addition, infection plays a crucial role in compromised wound healing [6, 7]. Contamination and infection of wounds can disrupt the regular healing sequence and result in a chronic inflammation, which hinders reepithelization [8–10]. Most chronic wounds are polymicrobial, and infections generally involve mixed populations of aerobic and anaerobic bacteria [11, 12]. *Staphylococcus aureus* is considered to be the most problematic germ in traumatic, surgical and burn wound infections [12, 13], but other microorganisms such as *Pseu-*

domonas aeruginosa, *Escherichia coli* and *Klebsiella pneumoniae* may also play a role in chronic wound infection [13]. Hence, an antimicrobial treatment, based on systemic and topical antibiotics or using antiseptic substances, is often necessary to avoid or to eliminate wound infection. As the widespread use of antibiotics was associated with the emergence of resistant bacterial strains such as MRSA, antiseptics have become an important alternative in antimicrobial applications. The most commonly used substances in clinical settings are povidone (PVP)-iodine, silver nitrate, chlorhexidine, octenidine and polihexanide [14, 15]. However, only limited knowledge exists on the pH dependency of the antibacterial efficacy of these substances. It is of interest to investigate the influence of the pH on the performance of antiseptics or antimicrobial wound dressings as it has been shown that the pH in chronic wounds most commonly has a range of 6.5–8.5 [16, 17]. This shift towards higher pH values in chronic wounds compared to acute wounds is called ‘alkaline shift’. The alkalization is thought to be due to both tissue necrosis and the presence of microorganisms. Therefore, establishing a low physiological pH might be a key factor to aid wound healing. In vitro studies have shown that wound dressings can have significant effects on the pH. Researchers found that shifts of up to 3 log values towards both the alkaline and acidic milieu are possible [18]. Conversely, a recent study by Braunwarth et al. [19] demonstrated that the antimicrobial effect of polihexanide-containing biocellulose wound dressings is pH-dependent, while silver-containing dressings possess similar bacteriostatic effects within a pH range of 5.5–9.0. These experiments were carried out using the agar diffusion test (ADT). Thus, the results do not only depend on the influence of the pH on the antibacterial activity but also on the diffusion capacity of the agent tested under different pH. To further investigate the influence of the pH on the activity of antimicrobial substances and wound dressings, it would be advantageous to determine microbial growth using microplate laser nephelometry (MLN). MLN presents a valuable tool to investigate pH influence on antimicrobial activity, as it allows high-throughput screening, incubation over a prolonged time period, and in situ monitoring of changes in the dose-response curves as well as the half maximal inhibitory concentration (IC₅₀) [20–23]. This study uses both ADT and MLN to investigate the influence of the pH on the activity and the efficacy of common antiseptic substances, such as silver nitrate, polihexanide, chlorhexidine, octenidine and PVP-iodine. *S. aureus* and *P. aeruginosa*, the most prominent bacteria in wound infection [12, 24], have been used

as model organisms. Moreover, the antibiotics vancomycin and gentamycin were included in the study for comparison.

Materials and Methods

Materials

The following antiseptic substances were selected for this study: silver nitrate (ACS reagent ≥99%, Sigma, St. Louis, Mo., USA), polihexanide (Cosmocil® CQ 20% polyhexamethylene biguanide, ARCH Chemicals, Rochester, N.Y., USA), chlorhexidine (chlorhexidine digluconate solution, 20% in H₂O, Sigma), octenidine (0.5% octenidine dihydrochloride concentrate, Schülke & Mayr GmbH, Norderstedt, Germany) and PVP-iodine (Sigma). The antibiotics vancomycin (hydrochloride, ≥900 IU/mg, Carl Roth GmbH, Karlsruhe, Germany) and gentamycin (sulphate, ≥590 IU/mg, Carl Roth GmbH) were used for comparison.

S. aureus ATCC 6538 and *P. aeruginosa* ATCC 27853 were purchased from the Deutsche Sammlung von Mikroorganismen und Zellkulturen, Germany. For cultivation of bacteria, special peptone and ‘lab-lemco’ powder for preparation of caso-bouillon and bacteriological agar were obtained from Oxoid (Basingstoke, UK). Columbia agar plates with 5% sheep blood were purchased from Biomerieux (Marcy l’Etoile, France), 0.9% NaCl solution was obtained from Fresenius Kabi Deutschland GmbH (Bad Homburg, Germany). Biodisks with gentamycin (10 µg) and vancomycin (30 µg) as well as additive-free disks were obtained from Biomerieux, and 1 M HCl and 1 M NaOH were purchased from Carl Roth GmbH.

Evaluation of Antibacterial Activity at Different pH by ADT

The ADT was performed in accordance with the DIN 58940-3. Caso-bouillon (pH 7.0) with different pH was prepared by addition of HCl, yielding caso-bouillon with pH 6.0 and 5.0, and by adding NaOH, producing pH 8.0 and 9.0, respectively. These media were supplemented with 1.5% agar, carefully heated until the agar was dissolved, and then cast into Petri dishes (Greiner Bio-One, Essen, Germany; 10 ml per dish) under sterile conditions. The prepared caso-agar plates were kept at 4°C until use. Twenty milliliters of caso-bouillon (pH 7.0) were inoculated with 1–2 colonies of the test organisms grown on Columbia agar plates and incubated for 24 h at 37°C under shaking. These *S. aureus* and *P. aeruginosa* cell suspensions were diluted 1:100 using the caso-bouillon with adjusted pH. These working suspensions had a microbial count of app. 1–5 × 10⁶ CFU/ml, and 100 µl of these suspensions were plated on the prepared caso-agar plates and left to dry for 10 min. Afterwards, the disks were placed onto the inoculated agar plates and wetted with 20 µl of each antiseptic. Negative controls (disks without additive) and positive controls (*S. aureus*: biodisks with 30 µg vancomycin and *P. aeruginosa*: biodisks with 10 µg gentamycin) were soaked with 20 µl 0.9% NaCl. All plates were incubated for 24 h at 37°C. The zone of inhibition (ZOI) was then measured in millimeters and all plates were photographed for documentation.

Determination of Efficacy of Antiseptics at Different pH by MLN

MLN was performed in accordance with National Committee for Clinical Laboratory Standards documents M27-A2 and DIN

EN 27027. Caso-bouillon (pH 7.0) with a different pH was prepared by adding HCl, yielding caso-bouillon with pH 6.0, and NaOH, producing pH 8.0 and 9.0, respectively. Twenty milliliters of caso-bouillon (pH 7.0) were inoculated with 1–2 colonies of the test organisms grown on Columbia agar plates and incubated for 24 h at 37°C under shaking. These *S. aureus* and *P. aeruginosa* cell suspensions were diluted 1:10⁵ in serial steps using the caso-bouillon with adjusted pH. These working suspensions had a microbial count of approx. 5×10^3 CFU/ml, and 100 µl of these suspensions were put in the respective wells of the 96-well microplate that contained the prepared dilutions of the antiseptics. Serial dilutions of the test substances were prepared in 0.9% NaCl; 100 µl each were put in triplicate into the respective wells of a sterile, clear, 96-well microplate (Greiner Bio-One). Blanks for each substance concentration tested were run at every assay. The microplates were covered with a clear adhesive film (Greiner Bio-One). The adhesive film was punctured with a 25-gauge needle at the right brim of the well to allow gas exchange. The microplates were then placed in the microplate laser nephelometer (NEPHELOstar Galaxy, BMG Labtech, Offenburg, Germany) and incubated for 24 h at 37°C. During incubation, the microplates were shaken in the instrument except during the hourly measurement. To determine the growth of the microorganisms, the turbidity was plotted against the incubation time for each antiseptic concentration tested. Subsequently, the 'area under the curve' was determined from the results and calculated as a percentage of the untreated control [bacterial growth (%)]. This was used to realize a dose-response curve for each antiseptic tested at the different pH from which the IC₅₀ of the antiseptics (under the experimental conditions used) was calculated, using a logistic fit function $[y = A2 + (A1 - A2)/(1 + (x/x0)^p)]$ where A1 is the upper limit, A2 is the lower limit, x0 is the IC₅₀ and p is the slope of the curve (Origin 7.5, OriginLab, Northampton, Mass., USA).

Statistical Analysis

One-way analysis of variance was carried out to determine statistical significance (Microsoft® Excel 2000). Differences were considered statistically significant at a level of $p < 0.05$. Asterisks indicate significant deviations from the control at the respective incubation time (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

Results

The pH Affects Bacterial Growth in Solution

MLN was used to monitor the growth of *S. aureus* and *P. aeruginosa* at different pH by the turbidity of the respective solution (fig. 1a, b). While turbidity requires relatively high concentrations of particles and obeys Beer's law, MLN uses a laser beam and measures its scattering by particles in solution at a right or forward angle. Compared to other methods used [25, 26], it enables high-throughput screening of several antiseptics at different pH in a short time. Furthermore, it allows the in situ recording of dose-response curves and the simultaneous monitoring of the IC₅₀ value [20, 21]. It was found that a

low pH (5.0) effectively inhibited microbial growth in solution. While no difference in the growth of *S. aureus* and *P. aeruginosa* was observed at pH 7.0–9.0, their progeny at pH 5.0 was found to be reduced to <10% of the control at pH 7.0 (fig. 1c).

The ADT was performed in accordance with the DIN 58940-3. In contrast to the experiments using MLN, bacteria did still grow on caso-agar plates with a low pH of 5.0. After 24 h, culture plates were completely covered with bacterial lawn at all pH values tested (fig. 2). As positive controls for ADT disks containing vancomycin (30 µg, *S. aureus*) and gentamycin (10 µg, *P. aeruginosa*) were used. Positive controls were effective at each pH tested; however, a significant influence on ZOI formation could be observed.

Determination of the Influence of the pH on Antibacterial Activity by ADT

Caso-agar plates with different pH values (5.0, 6.0, 7.0, 8.0 and 9.0) were inoculated with *S. aureus* (fig. 3a) or *P. aeruginosa* (fig. 3b). It could be noted that the antibiotics vancomycin and gentamycin used as positive controls also exhibited a pH-dependent activity. Here, bactericidal efficacy decreased with increasing pH for vancomycin (fig. 3a), while gentamycin (fig. 3b) showed enhanced effects at a higher pH.

Further experiments evaluated the influence of the pH on the antibacterial activity of the antiseptic substances selected (fig. 3). All substances were tested in concentrations that would be applied in clinical settings. The silver nitrate solution (0.1%) caused an average ZOI of 10 mm and was equally effective against *S. aureus* and *P. aeruginosa*. No changes in ZOIs were observed with different pH values. Chlorhexidine (0.2%) was able to inhibit the growth of *S. aureus* and *P. aeruginosa* in a similar fashion. No significant difference in the antibacterial efficacy was found between pH 6 and pH 9. Yet, at pH 5, a slightly higher activity was observed; however, a significant difference in the size of the ZOI at pH 5 compared to pH 7 was only observed for *S. aureus* ($p < 0.001$, *P. aeruginosa*: n.s.). Moreover, octenidine (0.1%) exhibited a pH-independent antibacterial activity against *S. aureus* and *P. aeruginosa* in vitro. In contrast, polihexanide (0.04%) exhibited increased efficacy against *S. aureus* with a rising pH. It was also found that the antibacterial activity of polihexanide against *P. aeruginosa* increased from pH 7 to pH 9. However, larger ZOIs were found at pH 5 and 6 compared to at pH 7. PVP-iodine (10%) showed a significant loss of antibacterial efficacy against both *S. aureus* and *P. aeruginosa* with a rising pH.

Fig. 1. Growth curves of *S. aureus* (a) and *P. aeruginosa* (b) at pH 5.0, 6.0, 7.0, 8.0 and 9.0 determined by MLN. c Bacterial growth at 37°C after 24 h at the respective pH values was compared to the medium control at pH 7.0. It was found that a low pH of 5.0

effectively inhibits the growth of *S. aureus* and diminishes *P. aeruginosa* progeny in solution. Asterisks indicate significant deviations from the control at pH 7.0 (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

Employing MLN to Evaluate the pH Effect on Antibacterial Activity

Although the ADT offers the possibility to observe changes in situ, it depends on the diffusion capacity of the active agent tested. Large molecules may have a reduced ability to disperse through the agar compared to smaller molecules, or their charge might impede diffusion. In both cases, the test outcome is influenced. As MLN inves-

tigations are performed in solution, they do not depend on the diffusion capacity of the substances and enable the direct measurement of interactions between substance and germs.

Results are summarized in figures 4 and 5. Because low pH (5.0) inhibited microbial growth in solution, MLN tests were only performed at a pH range of 6.0–9.0. At these pH values, no significant differences in the bacteri-

Fig. 2. Caso-agar plates with different pH were inoculated with *S. aureus* and *P. aeruginosa* and incubated at 37°C for 24 h. Plates were completely covered with a bacterial lawn at all pH values test-

ed. Positive (P) controls (*S. aureus*: vancomycin and *P. aeruginosa*: gentamycin) exhibited different effects depending on the pH. Negative (N) controls did not induce a ZOI.

cidal activity of vancomycin against *S. aureus* were observed. In contrast, the efficacy of gentamycin against *P. aeruginosa* increased significantly from pH 6.0 to pH 9.0. Moreover, it could be shown that most antiseptics possess a pH-dependent antimicrobial activity. For instance, a significant influence of the pH on the efficacy of silver nitrate, polihexanide and PVP-iodine was found in vitro. In addition, it was observed that the influence of the pH on the efficacy of the antiseptics differed among the bacteria strains tested. *S. aureus* exhibited an increasing sensitivity against both silver nitrate and polihexanide with a rising pH. It could be shown that the IC₅₀ values of polihexanide significantly decreased from 0.5 (at pH 6.0) to 0.05 µg/ml (at pH 9.0) and for silver nitrate from 2.1 (at pH 6.0) to 0.5 µg/ml (at pH 9.0). *P. aeruginosa* displayed an enhanced sensitivity only for polihexanide. Here, IC₅₀ values decreased from 0.6 (at pH 6.0) to 0.2 µg/ml (at pH 9.0). In contrast, 2.3-times more silver nitrate was necessary at a higher pH to achieve a similar growth reduction of *P. aeruginosa* in vitro. While both *S. aureus* and *P. aeruginosa* exhibited an increased sensitivity against polihexanide with a rising pH, the efficacy of PVP-iodine was significantly reduced. In particular, the ability of PVP-iodine to reduce the growth of *S. aureus* was markedly affected. This is in accordance with the ADT results. The antibacterial activity of the antiseptics chlorhexidine and

octenidine was only marginally affected by the pH in vitro. No significant changes were observed for chlorhexidine, while comparison of the IC₅₀ values for octenidine at different pH values revealed a slight but statistically significant decrease against *S. aureus* at pH 9.0.

Discussion

pH is defined as the negative logarithm of the activity of hydrogen ions in aqueous solution and is used to express acidity and alkalinity on a scale of 0–14. The pH of normal skin ranges from about 4.0 to 6.0 and has been recognized as the ‘acid mantle’ of the skin. It is of vital importance for the skin function and its resistance to external noxa [27]. The pH milieu in wounds has a direct as well as an indirect influence on many factors during wound healing. Studies have shown that the pH of chronic wounds most commonly has a range of 6.5–8.5 while the pH of acute, healing wounds is significantly lower [16, 17]. This alkalization or ‘alkaline shift’ is thought to be due to tissue necrosis and the presence of microorganisms. Hence, establishing a low physiological pH might aid wound healing [27]. Chemical acidification of the wound bed is thought to reduce bioburden as the growth of most human pathogens is inhibited at pH levels <6.0

Fig. 3. For the ADT, caso-agar plates with different pH were inoculated with *S. aureus* (a) or *P. aeruginosa* (b) and incubated with the antimicrobial substances at 37°C for 24 h. Afterwards, the ZOI was measured. Vancomycin and gentamycin were used as positive controls. The negative controls did not induce a ZOI. Asterisks indicate significant deviations from results at pH 7.0 (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

Fig. 4. Dose-response curves at different pH for the antibiotics vancomycin and gentamycin as well as for the antiseptic substances silver nitrate, polihexanide, PVP-iodine, chlorhexidine and octenidine against *S. aureus* (**a**) and *P. aeruginosa* (**b**) were recorded using MLN. (For figure 4b see next page.)

[17]. This could be confirmed by tests using MLN where acidic pH led to a significant decrease of bacterial growth. However, no significant effect on the growth of *S. aureus* and *P. aeruginosa* was observed on the agar plates at pH 5.0. This could indicate that the colonization of a surface enables bacteria to change the pH of their environment, e.g. the bacterial colonization of intact skin is accompanied by an increase of the local pH [17]. Therapeutic

prospects are clearly conceivable from these results. In vitro studies show that wound dressings can have significant effects on the pH [18]. However, the inverse question has to be raised, i.e. whether wound pH affects the activity and efficacy of antimicrobial agents. As chronic wounds are frequently colonized by different kinds of microorganisms, the most prominent being *S. aureus* and *P. aeruginosa* [12, 24], an antimicrobial

Fig. 5. The specific IC₅₀ were calculated from the dose-response curves recorded at different pH for the antimicrobial substances tested against *S. aureus* (a) and *P. aeruginosa* (b) using MLN. Asterisks indicate significant deviations from results obtained at pH 7.0 (* p < 0.05; ** p < 0.01; *** p < 0.001).

treatment is often necessary to avoid or eliminate wound infection. Hence, it is of interest to investigate the influence of the pH on the performance of antimicrobial substances. The results presented here show that most antiseptics as well as antibiotics possess a pH-dependent antimicrobial activity. The most pronounced influence on antibacterial efficacy was observed for polihexanide, PVP-iodine and gentamycin in vitro. However, while polihexanide and gentamycin displayed an increasing activity with rising pH, the antimicrobial effect of PVP-iodine was significantly reduced.

Polihexanide (fig. 6) exhibited an enhanced bactericidal efficacy against *S. aureus* and *P. aeruginosa* in the MLN measurements with increasing pH. As expected, the polycation was found to be more effective against the Gram-positive bacteria *S. aureus* compared to the Gram-negative bacteria *P. aeruginosa*. These differences in the bactericidal capacity can be explained by the differences in the cell wall properties of Gram-positive and Gram-negative bacteria [15]. In the ADT, polihexanide showed an increase of antibacterial activity against *S. aureus* in the

pH range of 5.0–9.0; for *P. aeruginosa*, a higher efficacy was also observed from pH 7 to pH 9. However, larger ZOI were found at pH 5.0 and pH 6.0 compared to at pH 7.0. This could be due to differences in the diffusion capacity of polihexanide at the respective pH values and/or might be caused by the particular features of *P. aeruginosa* at low pH values. The overall increase of the efficacy of polihexanide against both species with rising pH can be explained by its polycationic nature. At physiological pH, the positively charged groups of polihexanide can bind rapidly to the negatively charged surface of the bacteria. This causes membrane damage and the death of the bacteria [28]. In an alkaline solution, the biguanid groups present can still become protonated due to their pK_a value of 10.96, which leads to a higher charge density intensifying the binding to the bacterial surface [29] and maybe the higher antimicrobial activity observed.

Gentamycin (fig. 6) is an aminoglycoside antibiotic used to treat severe infections by Gram-negative bacteria. Its mechanism of action is to inhibit bacterial protein synthesis by binding to the 70S-ribosomal subunit and hin-

Fig. 6. Comparison of the chemical structures of vancomycin (**a**), gentamycin (**b**), silver nitrate (**c**), polihexanide (**d**), PVP-iodine (**e**), chlorhexidine (**f**) and octenidine (**g**). Asterisks indicate chain elongations by subunits depicted.

dering aminoacyl-tRNA transfer. At a higher pH, the uptake of gentamycin might be increased, as the alkalization of the environment also changes the bacterial membrane, leading to either more or less susceptibility to antimicrobial substances [15]. Moreover, it is known that the trisaccharide structure of gentamycin can be damaged by acidic treatment, causing inactivation of the aminoglycoside antibiotic at a lower pH [30].

In contrast, PVP-iodine, the complex of polyvinyl pyrrolidone and triiodine ions (fig. 6) with a broad microbicidal spectrum, is not active beyond pH 7 [31]. Hence, although iodine acts very quickly [32], a significant loss of its antibacterial efficacy can be observed, increasing the pH from 5.0 to 9.0. Here, similar results were obtained in both test systems for *S. aureus* and *P. aeruginosa*.

Results for vancomycin and silver nitrate differed between ADT and MLN. While vancomycin (fig. 6) displayed a significantly higher bactericidal effect at a lower pH in the ADT, no significant difference in the antibacterial activity was observed with MLN. Vancomycin is a large glycopeptide antibiotic used in the treatment of infections caused by Gram-positive bacteria. It acts by inhibiting their cell wall synthesis by forming bonds with the terminal D-alanyl-D-alanine moieties of the NAM/NAG-peptides, which prevents their elongation and cross-linking to fashion a solid cell wall. As MLN did not show significant differences in the bactericidal effect at the pH range tested, the increase of the antibacterial activity observed by ADT was most likely due to a different charge distribution and resulting differences in the diffu-

sion capacities of the antibiotic. In contrast, silver nitrate did not show a pH-dependent effect in the ADT but exhibited pH sensitivity during MLN. Unlike the antibiotic, the silver ion (Ag^+) works on multiple components of bacterial cell metabolism. Silver ions react with inorganic compounds, organic acids, proteins, DNA and RNA, killing the microorganisms through inhibition of cellular respiration, interference with DNA replication and alteration of cellular membrane permeability [8]. Besides, the diffusion capacity of the small silver ion is unlikely to be altered by pH in the ADT. Interestingly, it was observed that the influence of the pH on silver nitrate efficacy in MLN differs among bacteria. While *S. aureus* exhibited an increasing sensitivity against silver nitrate with a rising pH, about 2 times more silver nitrate was necessary at pH 9.0 compared to at pH 6.0 to achieve a similar growth reduction of *P. aeruginosa*. This might indicate that an alkaline environment has different effects on bacteria species [15]; it increases the susceptibility of *S. aureus* but it might strengthen the defiance of *P. aeruginosa*.

Like polihexanide, chlorhexidine and octenidine are positively charged (fig. 6) and exhibit their effect by interacting with negatively charged molecules in the bacterial cell membranes, resulting in their disruption. It could be shown that these positively charged antiseptics can induce the aggregation of acidic lipids in the vicinity of the adsorption site, which changes membrane permeability and may alter the function of membrane-associated enzymes, causing a leakage of cytoplasmatic compounds such as K^+ [15]. Moreover, the increased permeability of the cell wall allows small molecules like chlorhexidine and

octenidine to penetrate into the bacteria cell and act on targets within the bacteria [33]. Here, chlorhexidine and octenidine precipitated, for the most part, only slight changes in the antibacterial activity at different pH values in MLN as well as in ADT. It is most likely that the pH effect on these substances is less notable due to their lower molecular weight compared to the polycation polihexanide [29]. Hence, it is improbable that their charge density is altered with rising pH in the same way as polihexanide is affected.

In conclusion, the treatment of wound infections has an important role in wound management. A high bio-burden will disrupt the normal healing process and lead to development of persistent, nonhealing wounds [8–10]. The presence of bacteria further leads to the alkalization of the wound environment. It has been shown that the pH in chronic wounds most commonly has a range of 6.5–8.5 [16, 17]. This shift towards higher pH values in chronic wounds compared to acute wounds makes it imperative to know whether the antimicrobial efficacy of applied antimicrobial substances is altered by different pH levels. Our results suggest that the application of polihexanide might be advantageous for the management of wound infections, as both *S. aureus* and *P. aeruginosa* displayed an increased susceptibility with raised pH.

Acknowledgements

This work was partially supported by Lohmann & Rauscher GmbH & Co. KG (Germany). The authors would like to thank Denise Reichmann for excellent technical assistance.

References

- 1 Nwomeh BC, Yager DR, Cohen IK: Physiology of the chronic wound. *Clin Plast Surg* 1998;25:341–356.
- 2 Mast BA, Schultz GS: Interactions of cytokines, growth factors, and proteases in acute and chronic wounds. *Wound Rep Reg* 1996;4:411–420.
- 3 Barrick B, Campbell EJ, Owen CA: Leukocyte proteinases in wound healing: roles in physiologic and pathologic processes. *Wound Rep Reg* 1999;7:410–422.
- 4 Trengove NJ, Stacey MC, MacAuley S, Bennett N, Gibson J, Burslem F, Murphy G, Schultz G: Analysis of the acute and chronic wound environments: the role of proteases and their inhibitors. *Wound Rep Reg* 1999;7:442–452.
- 5 Yager DR, Nwomeh BC: The proteolytic environment of chronic wounds. *Wound Rep Reg* 1999;7:433–441.
- 6 Sibbald RG, Orsted H, Schultz GS, Coutts P, Keast D: Preparing the wound bed 2003: focus on infection and inflammation. *Ostomy Wound Manage* 2003;49:23–51.
- 7 Falanga V: Classifications for wound bed preparation and stimulation of chronic wounds. *Wound Rep Reg* 2000;8:347–352.
- 8 Warriner R, Burrell R: Infection and the chronic wound: a focus on silver. *Adv Skin Wound Care* 2005;18:2–12.
- 9 Kingsley A: The wound infection continuum and its application to clinical practice. *Ostomy Wound Manage* 2003;49:1–7.
- 10 Wright JB, Lam K, Buret AG, Olson ME, Burrell RE: Early healing events in a porcine model of contaminated wounds: effects of nanocrystalline silver on matrix metalloproteinases, cell apoptosis, and healing. *Wound Rep Reg* 2002;10:141–151.
- 11 Gjødtsbøl K, Christensen JJ, Karlsmark T, Jørgensen B, Klein BM, Krogfelt KA: Multiple bacterial species reside in chronic wounds: a longitudinal study. *Int Wound J* 2006;3:225–231.
- 12 Dissemont J, Schmid EN, Esser S, Witthoff M, Goos M: Bakterielle Kolonisation chronischer Wunden. *Hautarzt* 2004;55:280–288.
- 13 Bowler PG, Duerden BI, Armstrong DG: Wound microbiology and associated approaches to wound management. *Clin Microbiol Rev* 2001;14:244–269.
- 14 White RJ, Cutting K, Kingsley A: Topical antimicrobials in the control of wound bioburden. *Ostomy Wound Manage* 2006;52:26–58.
- 15 McDonnell G, Russell AD: Antiseptics and disinfectants: activity, action and resistance. *Clin Microbiol Rev* 1999;12:147–179.

- 16 Eberlein T: Experience in Europe. 12th Congress of the German Society of Woundhealing, Freiburg, 2010.
- 17 Dissemond J, Witthoff M, Brauns TC, Haber D, Goos M: pH-Wert des Milieus chronischer Wunden. *Hautarzt* 2003;54:959–965.
- 18 Weindorf M: pH-Werte moderner Wundauflagen und deren Einfluss auf das umgebende Milieu: Resultate einer in vitro Untersuchung. *ZfW* 2007;12:92–97.
- 19 Braunwarth H, Brill FHH, Brill H: Results of in vitro testing of wound dressings with sustained release of polyhexanide (PHMB) and silver-ions at different pH-values. *Wound Manage* 2011;3:119–125.
- 20 Finger S, Wiegand C, Buschmann H-J, Hipler U-C: Antimicrobial properties of cyclodextrin-antiseptics-complexes determined by microplate laser nephelometry and ATP bioluminescence assay. *Int J Pharm* 2012;436:851–856.
- 21 Wiegand C, Abel M, Ruth P, Hipler U-C: Analysis of the adaptation capacity of *Staphylococcus aureus* to commonly used antiseptics by microplate laser nephelometry. *Skin Pharmacol Physiol* 2012;25:288–297.
- 22 Seyfarth F, Schliemann S, Elsner P, Hipler U-C: Antifungal effect of high- and low-molecular-weight chitosan hydrochloride, carboxymethyl chitosan, chitosan oligosaccharide and N-acetyl-D-glucosamine against *Candida albicans*, *Candida krusei* and *Candida glabrata*. *Int J Pharm* 2008;353:139–148.
- 23 Fouda MMG, Knittel D, Hipler U-C, Elsner P, Schollmeyer E: Antimycotic influence of β -cyclodextrin complexes – in vitro measurements using laser nephelometry in microtiter plates. *Int J Pharm* 2006;311:113–121.
- 24 Kirketerp-Møller K, Jensen PO, Fazli M, Madsen KG, Pedersen J, Moser C, Tolker-Nielsen T, Høiby N, Giuskov M, Bjørnsholt T: Distribution, organization, and ecology of bacteria in chronic wounds. *J Clin Microbiol* 2008;46:2717–2722.
- 25 Walsh SE, Maillard J-Y, Russell AD, Cattranich CE, Charbonneau DL, Bartolo RG: Development of bacterial resistance to several biocides and effects on antibiotic susceptibility. *J Hosp Infect* 2003;55:98–107.
- 26 Suller MTE, Russell AD: Antibiotic and biocide resistance in methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant *Enterococcus*. *J Hosp Infect* 1999;43:281–291.
- 27 Dissemond J: Die Bedeutung des pH-Wertes für die Wunde. *Hartmann Wundforum* 2006;1:15–19.
- 28 Ikeda T, Tazuke S, Watanabe M: Interaction of biologically active molecules with phospholipid membranes. I. Fluorescence depolarization studies on the effect of polymeric biocide bearing biguanide groups in the main chain. *Biochim Biophys Acta* 1983;735:380–386.
- 29 Timofeeva L, Kleshcheva N: Antimicrobial polymers: mechanism of action, factors of activity, and applications. *Appl Microbiol Biotechnol* 2011;89:475–492.
- 30 Gesellschaft für Dermopharmazie e.V.: Wirkstoffdossiers für externe dermatologische Rezepturen, 2007.
- 31 Ratiopharm GmbH: Fachinformation PVP-Jod-ratiopharm® Salbe, 2008.
- 32 Reimer K, Wichelhaus TA, Schäfer V, Rudolph P, Kramer A, Wutzler P, Ganzer D, Fleischer W: Antimicrobial effectiveness of povidone-iodine and consequences for new applications. *Dermatology* 2002;204:114–120.
- 33 Mohammadi Z, Abbott PV: The properties and applications of chlorhexidine in endodontics. *In Endodont J* 2009;42:288–302.