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ORIGINAL ARTICLE

Effectiveness of a polyhexanide irrigation solution on methicillin-resistant *Staphylococcus aureus* biofilms in a porcine wound model

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Abstract

Irrigation and removal of necrotic debris can be beneficial for proper healing. It is becoming increasingly evident that wounds colonized with biofilm forming bacteria, such as *Staphylococcus aureus* (SA), can be more difficult to eradicate. Here we report our findings of the effects of an irrigation solution containing propyl-betaine and polyhexanide (PHMB) on methicillin-resistant *Staphylococcus aureus* (MRSA) biofilms in a porcine wound model. Thirty-nine deep partial thickness wounds were created with six wounds assigned to one of six treatment groups: (i) PHMB, (ii) Ringer's solution, (iii) hypochlorous acid/sodium hypochlorite, (iv) sterile water, (v) octenidine dihydrochloride, and (vi) octenilin. Wounds were inoculated with MRSA and covered with a polyurethane dressing for 24 hours to allow biofilm formation. The dressings were then removed and the wounds were irrigated twice daily for 3 days with the appropriate solution. MRSA from four wounds were recovered from each treatment group at 3 days and 6 days hours after initial treatment. Irrigation of wounds with the PHMB solution resulted in 97·85% and 99·64% reductions of MRSA at the respective 3 days and 6 days assessment times when compared to the untreated group. Both of these reductions were statistically significant compared to all other treatment groups (*P* values <0·05).

Introduction

Chronic wounds are becoming an increasingly common health malady around the world. In the United States alone, there are currently around 6·5 million people suffering from chronic wounds (1). In an effort to treat these afflictions, Americans spend over $25 billion yearly (2). Significant morbidity and mortality are associated with chronic wounds. Similar to many forms of cancer, diabetic foot ulcer complications have been reported to result in 5-year mortality rates (3). In a 2-year study evaluating the mortality of patients with various chronic wound types, 28% of those examined as outpatients died (4). These data demonstrate a dire need for the investigation of factors responsible for impeded wound healing.

There are many possible intrinsic and extrinsic components that can slow the healing process; however a leading cause is bacterial infection. Without an epidermal barrier, hosts are especially susceptible to endogenous and exogenous microbial colonisation (5). One study reports that over 80% of leg ulcers contain bacteria (6). Once inside wounds, bacteria trigger a host inflammatory response that can impede the normal healing process if it persists (7). Specifically, bacteria and their

Key Messages

- wounds colonised with biofilm-forming bacteria are more difficult to eradicate, and new therapies are needed
- the aim of this study was to evaluate the benefit of a polyhexanide irrigation solution to remove MRSA from wounds compared to other available products
- a polyhexanide was able to reduce MRSA by over 3 log CFU/g as compared to sterile water irrigation
- compared to Ringers irrigation solution, the polyhexanide solution was able to reduce MRSA counts by ~2·5 log CFU/g

endotoxins are recognised by innate immune cells like macrophages, which upregulate proinflammatory cytokines like TNF-α and interleukin-1. Even if a small amount of bacterial cells manage to evade the host's methods of eradication, the immune response persists, thus altering keratinocyte stimulation and the proliferation stage of the healing process. It also causes elevated levels of matrix metalloproteases (MMPs), which continue to degrade the extracellular matrix (ECM) (8). The result is a chronic wound that will not heal without proper intervention.

Modern wound care strategies take advantage of a detailed set of principles known as 'TIME', an acronym for Tissue, Infection/Inflammation, Moisture and Edge. TIME dictates that necrotic tissue, consisting of dead cells, debris and bacteria, provides an ideal medium for infection and increased inflammation (9). As increased inflammation and infection often result in delayed healing, it is essential to manage necrotic tissue by cleansing the wound (10). Once the wound has been properly debrided, TIME principles mandate that the wound be kept moist in order to encourage healing. The final aspect of TIME refers to the migrating epidermis or wound 'Edge'. While debridement, control of infection, inflammation and management of moisture do not guarantee that the wound edge will migrate normally, they represent essential components of wound bed preparation that may encourage edge migration and healing (11).

Wound irrigation is often used in order to mildly debride wounds as mechanical debridement is not always necessary. Often times, irrigation is sufficient to dislodge foreign debris, loosely attached bacteria and damaged ECM. Normal saline or Ringer's solution are most frequently used to irrigate both acute and chronic wounds (12). These solutions are relatively cost effective and require no preparation by the clinician because of their availability. As they contain no antimicrobials and are isotonic, they are minimally toxic to exposed tissue and less likely to impede normal wound healing (13).

Saline and Ringer's solution, while both mild and readily available, may not always be the best choices for wound irrigation; however, bacteria such as methicillin-sensitive *Staphylococcus aureus* (MSSA) and methicillin-resistant *Staphylococcus aureus* (MRSA) are frequently found in chronic wounds and are capable of adopting a biofilm phenotype (14). Biofilms are bacterial colonies that have been encased in an extracellular polysaccharide matrix (EPS) of bacterial, host or mixed origin. Bacteria in the biofilm phenotype exist in an altered metabolic state within the protective EPS covering. The result is a unified slime attached to the host that is often not washed away by Ringer's solution or normal saline and has a 10–1000-fold increase in resistance to systemic and topical antibiotics (15,16). In addition, the close proximity of cells within the biofilm facilitates lateral gene transfer, which aids the passage of resistance genes from cell to cell and results in a more uniformly resistant population (17,18).

It is increasingly evident that biofilms have an enormous impact on medical treatment and health care costs. Estimates suggest that over 65% of nosocomial infections are related to biofilms (19). Because of the resistance, these infections have to be treated with traditional antibiotics and rinse solutions. They often result in additional trauma and severe complications that lead to longer, more intensive hospital stays and even death. Consequently, the monetary impact on the U.S. health care system of biofilm-related infections has been estimated to exceed $1 billion USD annually (20). The development of novel forms of eradication that overcome the defence mechanisms of biofilms, including mild wound irrigation solutions, has therefore become a recent topic of interest.

An irrigation solution of propyl-betaine (undecylenamidopropyl betaine) and polyhexanide (polyaminopropyl biguanide) is intended for cleansing and hydrating chronic wounds to assist in the management of superficial primary and secondary cutaneous infections. The active agents have been successfully used in other products for disinfection and preservation. Propyl-betaine is a mild surfactant found in cosmetic formulations for skin, hair cleansing and conditioning (21). Polyhexanide has been used as a disinfectant in swimming pools to control contamination with various amoeboid and bacterial organisms, including *Pseudomonas aeruginosa* (22–26). Additionally, it is commonly found in no-rinse products for cleansing contact lenses, including products marketed for use with 'sensitive-eyes' (27–29). The combination of the two chemicals has been shown to be well tolerated on the skin, as well as capable of significantly reducing *Pseudomonas aeruginosa* biofilm concentration after an observation time of 24 hours (30). In this study, we aimed to determine the effects of the PHMB wound irrigation solution on MRSA biofilms using a porcine wound model.

Materials and methods

Experimental animals

The following experiment was submitted to and approved by the University of Miami Animal Use Committee. The procedures followed the federal guidelines for the care and use of laboratory animals (U.S. Department of Health and Human Services, U.S. Department of Agriculture). This study was conducted in compliance with the University of Miami's Department of Dermatology & Cutaneous Surgery's Standard Operating Procedures (SOPs). Swine were used as our experimental research animal as their skin is morphologically similar to human skin (31). Three female-specific pathogen-free animals, weighing 35–45 kg, were kept in house for 2 weeks prior to initiating the experiment in order to acclimatise to the environment. The animals were fed a basal diet *ad libitum* and housed individually in our animal care facilities (American Association for Accreditation of Laboratory Animal accredited) with controlled temperature (19-21°C) and lights (12 hour/12 hour LD).

Animal preparation and wounding technique

The animals were anaesthetised for all procedures with Telazol (1·4 mg/kg), Xylazine (2 mg/kg), Atropine (0·05 mg/kg) I.M. and inhalation of an isoflurane/oxygen combination. After anaesthetising the pigs on the first day of surgery, the hair on the flanks and backs of the pigs was clipped with standard animal clippers. The skin on both sides of the animals was then

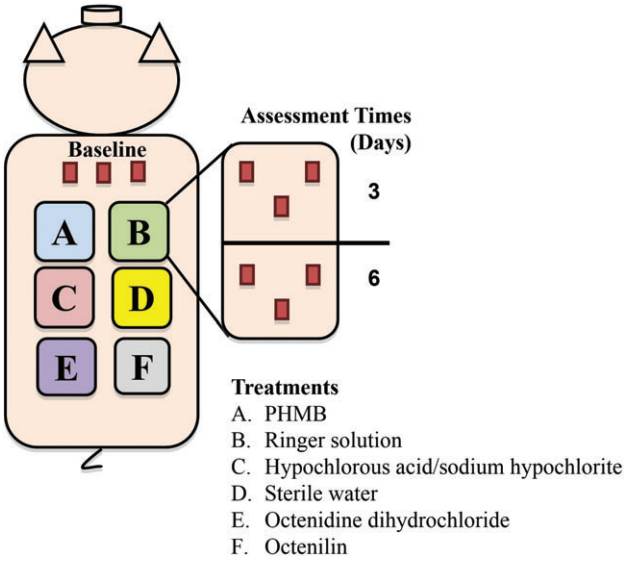

Figure 1 Experimental Design

prepared by washing with a non-antibiotic soap (Neutrogena®) and sterile water.

A total of 39 deep partial thickness wounds measuring 10 mm × 7 mm × 0·5 mm deep were made on the paravertebral and thoracic area of each animal using a specialised electrokeratome. Six wounds were assigned to six different treatment groups (Figure 1). Three wounds from each group were recovered on days 3 and 6. Wounds were separated from one another by 4–6 cm of unwounded skin. Analgesics were given during the experiment to prevent any discomfort.

Wound inoculation

A fresh culture of pathogenic isolate of ATCC 33593 (MRSA), obtained from the American Type Culture Collection, was used in these studies. The frozen bacterium was recovered from glycerol stock [15% glycerol in tryptic soy broth (TSB), −80°C]. All inoculum suspensions were made by scraping the overnight growth from a culture plate into 5 ml of normal saline. This resulted in a suspension concentration of approximately 10^8 colony-forming units/ml (CFU/ml). The 10^8 CFU/ml suspension was serially diluted to make an inoculum suspension with a concentration of 10^6 CFU/ml as determined by optical density at 570 nm. A small amount of the inoculum suspension was plated onto culture media to quantify the exact concentration of viable organisms. A 25-µl aliquot of this suspension was deposited into the centre of each wound. Each aliquot was then lightly scrubbed into the test site for 10 seconds using a sterile Teflon spatula and left for 3 minutes prior to covering the wounds with a polyurethane film dressing (each wound was dressed individually). Wounds remained covered for 24 hours to allow for the establishment of biofilms prior to treatment.

Treatment regimen

After the 24-hour biofilm formation period, the dressings were removed. A sterile, metal cap measuring 1·5′ in diameter was placed over each wound site, and a skin marker was used to encircle each treatment area. During each treatment, three of the four wounds in each group were covered with these caps to prevent the rinse from flowing onto the other wounds. Each wound was irrigated twice with one of the following treatment groups: (A) PHMB solution [Prontosan®, B Braun Medical, Melsungen, Germany], (B) Ringer's solution [Ringer B. Braun, Melsungen, Germany], (C) hypochlorous acid/sodium hypochlorite [Microdacyn$_{60}$®Wound Care, Oculus Technologies of Mexico, Zapopan, Jalisco, Mexico], (D) Sterile water, (E) octenidine dihydrochloride [Octenisept® farblos/incolore, Schulke & Mayr GmbH, Norderstedt, Germany] and (F) Octenilin [Octenilin®, Schulke & Mayr GmbH, Norderstedt, Germany]. Irrigation was performed twice daily using 10-ml syringes without needles. A syringe was held at a 45-degree angle over each site, and the entire wound area (1·5-inch diameter circle) was irrigated using constant pressure (approximately 20 psi). After irrigation, any excess fluid was blotted dry with sterile gauze without disturbing the wound, and each wound was covered separately with polyurethane dressing.

Bacterial recovery from wounds

On days 3 and 6, post-treatment biopsies were taken from three wounds in each treatment group. A punch biopsy (6 mm) was used to recover wounds for MRSA counts.

Microbiology

Biopsies were weighed and immediately placed in 1 ml of All Purpose Neutralizing Solution (containing tween 80, lecithin, sodium oleate, sodium thiosulfate, protease peptone and trypton) followed by the homogenisation in a sterile homogenisation tube (Tenbroeck Tissue Grinder, designed to gently homogenise tissues by mechanical shear). The sample was then combined with an additional 4 ml of Neutralizing Solution for serial dilutions. Serial dilutions were made from all culture samples, and the extent of microbiological contamination was assessed using the Spiral Plater System (Spiral Biotech, Norwood, MA). This system deposits a 50-µl aliquot of the scrub bacterial suspension over the surface of a rotating agar plate. Oxacillin Resistance Screening Agar (ORSAB, Oxoid LTD, Basingstoke, Hampshire, England) was used to isolate MRSA USA300. All plates were incubated aerobically overnight (24 hours) at 37°C, after which the number of viable colonies were counted. This method has been used for over 30 years to evaluate the antimicrobial efficacy of various topical agents and/or dressings (32–39). During this recovery process, both planktonic- and biofilm-associated bacteria are being assessed (38).

The harvested bacterial suspensions were serially diluted and cultured on a solid selective medium (ORSAB) for determination of the colony-forming units per ml (CFU/ml) of the recovery solution. All data from all three animals were combined and tabulated. Statistical analysis using nine samples per treatment group per assessment was analysed for significance using an ANOVA.

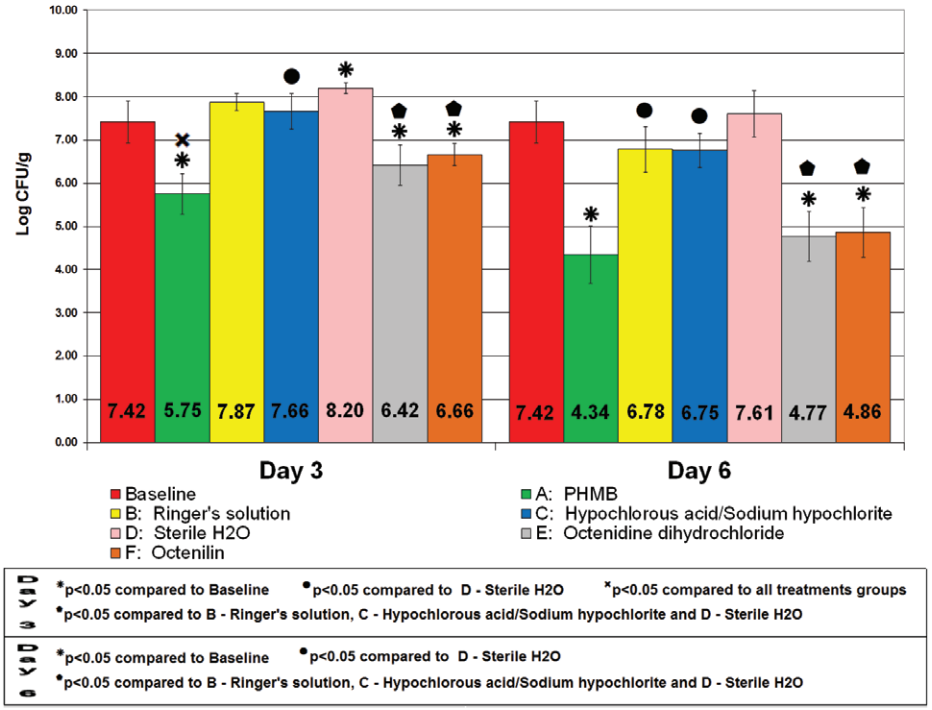

Figure 2 Combined bacterial counts of methicillin-resistant *Staphylococcus aureus* USA300 after treatment application.

Results

After 24 hours of biofilm formation, the wounds from the baseline wounds were recovered. The results showed an initial bacterial count of $7{\cdot}42 \pm 0{\cdot}49$ Log CFU/g. On day 3, wounds treated with PHMB exhibited the greatest amount of reduction in bacterial counts with $5{\cdot}75 \pm 0{\cdot}47$ log CFU/g (Figure 2). These wounds had bacterial reductions of 97·85% and 99·64% when compared against the baseline wounds and sterile water, respectively. The results of wounds treated with PHMB were significantly ($P < 0{\cdot}05$) lower when compared against all of the treatments groups. These wounds from the PHMB group exhibited better results than Ringer's solution, hypochlorous acid/sodium hypochlorite and sterile water by having a difference of at least 1·90 log CFU/g.

On day 3, wounds treated with both Ringer's Solution and hypochlorous acid/sodium hypochlorite resulted in similar results by having $7{\cdot}87 \pm 0{\cdot}20$ and $7{\cdot}66 \pm 0{\cdot}41$ log CFU/g, respectively (Figure 2). Hypochlorous acid/sodium hypochlorite had a significantly ($P < 0{\cdot}05$) lower CFU count than sterile water. The sterile water group exhibited the highest amount of MRSA at $8{\cdot}20 \pm 0{\cdot}13$ log CFU/g when compared against all other treatment groups on day 3. Ringer's solution-, hypochlorous acid/sodium hypochlorite- and sterile water-treated wounds had higher amounts of bacterial count than the baseline wounds on day 3. Wounds treated with Octenidine dihydrochloride and Octenilin on day 3 showed bacterial counts of $6{\cdot}42 \pm 0{\cdot}46$ and $6{\cdot}66 \pm 0{\cdot}26$ log CFU/g, respectively, which yields significantly ($P < 0{\cdot}05$) lower bacterial counts than Ringer's solution, hypochlorous acid/sodium hypochlorite and sterile water. Both Octenidine dihydrochloride and Octenilin treatment groups had significant ($P < 0{\cdot}05$) bacterial reductions of 90·0 and 82·6%, respectively, when compared against baseline wounds.

On day 6, PHMB significantly ($P < 0{\cdot}05$ compared to baseline) reduced the bacterial count at $4{\cdot}34 \pm 0{\cdot}67$ log CFU/g (99·92% bacterial reduction). PHMB had the lowest MRSA bacterial count on day 6 among all treatment groups and a bacterial reduction of 99·95% when compared against sterile water. Ringer's solution and hypochlorous acid/sodium hypochlorite showed significantly ($P < 0{\cdot}05$) lower rates than those treated with sterile water, with a bacterial count of $6{\cdot}78 \pm 0{\cdot}53$ and $6{\cdot}75 \pm 0{\cdot}40$ log CFU/g, respectively. Sterile water-treated wounds showed a higher bacterial count, $7{\cdot}61 \pm 0{\cdot}54$ log CFU/g, on day 6, compared to baseline wounds. Octenidine dihydrochloride and Octenilin showed results similar to PHMB in the amounts of MRSA at $4{\cdot}77 \pm 0{\cdot}58$ and $4{\cdot}86 \pm 0{\cdot}58$ log CFU/g, respectively (significantly lower than Ringer's Solution, hypochlorous acid/sodium hypochlorite and sterile water). Additionally, both Octenidine dihydrochloride and Octenilin had significantly ($P < 0{\cdot}05$) lower bacterial counts than baseline wounds, with bacterial reductions of 99·78% and 99·73%, respectively.

The treatment group with the most efficient bacterial reductions on both days 3 and 6 was PHMB. These wounds harboured the least amount of MRSA USA300 on both assessment days when compared against every treatment group for this study. On day 6, treatment groups Octenidine dihydrochloride and Octenilin showed results similar to the wounds treated with PHMB These groups were able to significantly reduce MRSA proliferation on both days 3 and 6 when compared against the baseline wounds, with PHMB being the most effective. Ringer's solution, hypochlorous acid/sodium hypochlorite and sterile water

provided the highest bacterial counts, with the latter harbouring more MRSA than the baseline wounds on both assessment days.

Discussion

The significant decreases of MRSA in the wounds treated with the PHMB solution provide encouragement for its viability as a wound rinse that is superior to more classical products. Such data correspond with those from an *in vitro* study in which three treatments of the same solution reduced MRSA growth by more than log 5 upon assessments at 7, 14 and 28 days after exposure (40). Although a reduction of this magnitude was not observed in our experiment, the PHMB solution was the only treatment to continually result in reduced bacterial loads. The normal saline and Ringer's solutions, despite producing reductions in MRSA microcolonies when compared to the untreated wounds, were unable to keep replication at bay, and the populations within wounds continued to expand. Infection, defined by the existence of proliferating foreign organisms within a wound after injury (41), was thus retarded only by the PHMB irrigation solution. In a clinical study involving the treatment of chronic leg ulcers of 40 patients with either the PHMB solution or normal saline for 4 weeks, a significantly lower pH was found in wounds treated with the former rinse. Moreover, the pH of the wounds treated with the PHMB combination continually decreased over the weeks of treatment, while the normal saline-treated wounds held a fairly consistent pH (42). Because elevated wound pH is a direct indicator of bacterial colonisation, the continued increases in acidity, along with a reduction in pain associated with the wounds, provide additional evidence that the PHMB irrigation solution is capable of reducing the bacterial load and possibly eradicating infection with continuous use.

One factor that may impede the decontamination capabilities of normal saline and Ringer's solution is the presence of biofilm. When bacteria are bound to a wound bed, the organisms often secrete an EPS, which serves as a protective covering as well as an apparatus through which they can communicate using signalling molecules in a process called quorum sensing. This method of communication allows the population to adapt to variations in the environment, like nutrient availability, thereby improving their ability to survive (43,44). Other consequences include the spread of virulence factors and expedited resistance development caused by lateral gene transfer between the closely encased microbes of various species (17,18). Overall, these factors combined with the glycocalyx barrier formed by the EPS make the population of prokaryotes substantially more resistant to antibiotics, lysosomal enzymes of phagocytes and conventional rinses (15,16,45). A frequent result is a persistent wound infection often attributed to impeded healing. Unrelenting levels of bacteria in the tissue continue to stimulate white blood cells to release proinflammatory cytokines, which consequently promote the prolonged breakdown of tissue through MMPs and decreased production of growth factors (46–48). Biofilms are therefore commonly associated with chronic wounds (49), making them of great clinical significance.

In order to test the PHMB irrigation solution against biofilms, we left the MRSA inoculum undisturbed within the wounds for 24 hours prior to treatment to achieve the desired phenotype. The solution's ability to reduce bacterial counts may be because of the inherent qualities of the two chemicals working in combination. The polyhexanide portion of the new irrigation solution is an antimicrobial agent similar to antimicrobial peptides produced naturally in the body responsible for denaturing the acidic lipid membranes of bacteria (50–52). Instead of simply rinsing away bacteria like the saline and Ringer's solutions, this component is able to eliminate MRSA by cell lysis. The glycocalyx formed by the EPS barrier, however, prevents polyhexanide from coming into direct contact with the bacterial cell membranes. As a result, the propyl-betaine portion of the combination is equally important in infection removal. This surfactant is not only able to envelope and wash away wound debris, but has been reported to disrupt biofilms. By doing so, polyhexanide can reach the foreign cells and lyse their membranes, consequently reducing the level of infection (53).

The demand for antiseptics capable of reducing infection is becoming increasingly high with the continued evolution of bacterial resistance to antibiotics (54,55). Novel resistance is generated by chromosomal mutation that allows a bacterium to avoid the destructive mechanisms of an antibiotic. As mentioned above, these genes can be transferred laterally between species of bacteria, resulting in the capacity to resist multiple antibiotics. As new antibiotics are introduced, bacteria continue to develop ways to evade their effects. Ultimately, strains can become untreatable by the available classes of antibiotics (56). MRSA is resistant to all β-lactam antibiotics, which function by inhibiting the synthesis of the peptidoglycan cell wall (57). Now, because the use of these drugs is believed to promote colonisation (58), other antibiotics like vancomycin have become the preferred treatments. However, as different strains of MRSA arise demonstrating resistance to these drugs as well, new therapies are needed (59,60). MRSA is thus more and more difficult to treat and has become one of the most prevalent causes of wound infection (61,62). Our results demonstrating MRSA's susceptibility to the PHMB antiseptic are promising evidence for an emerging treatment combination that can have a large impact on this resilient type of bacterial infection. Moreover, there have been no known signs of resistance to solutions containing the polyhexanide component responsible for cell lysis, making it a possible long-term solution (63,64).

Other bacteria commonly found in chronic wounds include *Enterococci*, *Enterobacteriaceae*, coagulase-negative *Staphylococci* and *Pseudomonas* (51,52). *Pseudomonas aeruginosa* is one of the more feared species of these pathogens because of its ability to produce a virulence factor called 'exotoxin A', which causes host cell necrosis through the inhibition of protein synthesis (65). Additionally, the Gram-negative bacterium is difficult to eradicate using antibiotics because its lipopolysaccharide envelope prevents drug permeation, and it can rapidly develop different efflux pumps that actively transport antimicrobial toxins outside of the cystoplasm (66). Polyhexanide, on the other hand, has been shown to be effective against *P. aeruginosa* biofilms *in vitro* (67,68). Moreover, polyhexanide was witnessed to kill *P. aeruginosa* isolates from chronic venous ulcers suspended in wound fluid *ex-vivo* and reverse the degradation of AMPs and human skin by inhibiting an elastase secreted by the bacterium (69). It has also been reported to be effective

against other pathogens like *Enterococcus faaesalis*, *Streptococcus pneumoniae*, *Escherichia coli*, *Clostridium perfingens*, *Haemphulus influenze*, *Candida albicans* and Human Immunodeficiency Virus (70,71). Such efficacy against a broad spectrum of infectious organisms, including Gram-positive and Gram-negative bacteria, fungi and viruses, provides further support for the use of the PHMB solution to reduce any type of pathogen burden within wounds.

The increasing demand for the wound antimicrobials alternative to antibiotics has given rise to more antimicrobial agents. There now exist dozens of products from various classes of compounds, including iodine, hydrogen peroxide, acetic acid, chlorohexidine and silver (72) With such a large array of treatments to chose from, it is important to not only assess their cytotoxicity to pathogenic cells but to the host's as well. A drawback to the broad-spectrum qualities of antiseptics is that they often have little to no specificity when it comes to discerning between the pathogen cells and those of the host. This means that many antiseptics with high efficacy against infectious pathogens also cause host cell death and impaired tissue regeneration. For instance, one study examining the effects of octenidine dihydrochloride, PHMB and providon iodine on *P. aeruginosa*-inoculated burn wounds in rats determined Octenidine dihydrochloride as the most effective antimicrobial of the three in eschar, muscle lung and blood tissues (73). However, another experiment analysing the effects of oxtenidine versus polyhexanide on the cicatrisation of aseptic piglet wounds showed that wounds exposed to the polyhexanide treatment closed more than 5 days earlier than those that received octenidine (74). The biocompatibility index (BI) is thus a measure more indicative of an antiseptic's efficacy than just its ability to reduce bioburden. The BI compares a compound's *in vitro* cytotoxity to the microbicidal effect. If the compound yields a BI > 1, it is considered an effective antimicrobial with negligible toxicity to the host. When tested against both *Staphyloccocus aureus* and *Escherichia coli*, polyhexanide has demonstrated BIs of 1·36 and 1·51, respectively. Furthermore, these values were notably higher than those of benzalkonium chloride, cetylpyridinium chloride, chlorohexadine digluconate, mild silver protein, providone iodine, silver nitrate, silver sulfadiazine and triclosan (75). Such data suggest that polyhexanide is a treatment superior to those aforementioned when comparing antiseptics that will not only cleanse wounds but allow for proper healing.

Results from wound-healing studies confirm polyhexanide's ability to kill bacteria while preserving host tissue. Wiegand *et al.* (76) demonstrated *in vitro* that keratinocytes, initially under siege from the effects of *S. aureus* in co-culture, were no longer damaged and could once again proliferate when treated with polyhexanide. Another study involving four patients presenting poorly healing decubitus ulcers revealed histological data illustrating that mesh grafts treated with polyhexanide were more effective at reducing necrosis and oedema while promoting epithelialisation than those soaked in silver nitrate or PVP–iodine solutions. When the authors conducted an additional trial assessing the efficacy of polyhexanide on second-degree burn wounds untreatable by skin grafts, the burns completely healed 10 days after a single debridement with the antiseptic (77). Data from a retrospective study involving the analysis of records from 59 patients with venous leg ulcers treated with either saline, Ringer's or the same PHMB solution used in our experiment further corroborates polyhexanide's antimicrobial attributes and low cytotoxicity to host tissue. The researchers concluded that the wounds rinsed with PHMB healed after an average of 3·31 months, whereas the saline-/Ringer's-treated wounds took around 4·42 months to completely close (78). The PHMB solution has even been shown to be the ideal therapeutic option to clean, decontaminate and maintain the optimum conditions for a number of wound types. In a case involving a 61-year-old patient afflicted with Fournier's gangrene, doctors were able to decontaminate and heal the wound after 58 days of treatment with the PHMB solution and occlusive dressings (79). Studies of leg ulcers and pressure ulcers showed higher efficacy of the propylbetaine–polihexanide solution in reducing inflammatory signs and accelerating the healing (80,81).

We have previously shown that debridement alone cannot adequately remove MRSA biofilms from wounds *in vivo*, suggesting that additional therapies are needed to control wound bioburden (82). The authors note that there are limitations of extrapolating preclinical efficacy into the clinical setting, especially as patients have different wound ideologies, sizes, depths and other variables. However, aggregating our current preclinical results with others, supporting the PHMB solution's potent antimicrobial properties, low host cell cytotoxicity, broad-spectrum activity, ability to perforate biofilms and minimal susceptibility to pathogen resistance creates a strong case for the use of the PHMB rinse to decontaminate and promote healing within wounds.

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