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The Scientific Bases for the Use of Hypochlorous Acid to Avoid Pitfalls

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Introduction

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Different agents for cleansing wounds have been reported since antiquity. Originally, it was necessary to remove foreign bodies and debris from wounds. After the discovery of bacteria and the development of the germ theory of disease in the 1860s, the removal of pathogens also became desirable. The problem with early agents for wound cleansing, which still remains today, is that many agents are injurious to the wound tissue and actually impede wound healing.

Hypochlorous acid (HOCl), a naturally occurring small molecule generated by white blood cells during the oxidative burst, essentially can kill all human pathogens and shows no negative effects on keratinocytes or fibroblasts. The agent with the highest percentage of pure HOCl is Vashe Wound Solution (Urgo Medical, Fort Worth, TX). Since its introduction several years ago, it has rapidly gained popularity and usage. Although there are a large number of scientifically sound reports of the effects of this product in the literature, greater usage leads to some examples of use beyond the data supporting the product.

In this supplement, the panel will discuss the scientific bases supporting the use of Vashe Wound Solution and point out pitfalls that may occur when the product is used in situations that have no scientific support.

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History of Hypochlorous Acid and Its Mechanism of Action

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In the latter half of the 19th century, scientists such as Pasteur, Lister, and Koch formalized the germ theory of disease. Koch conclusively demonstrated that bacteria could cause a specific disease response.[1] A major advance in the prevention and management of infection in the surgical patient was the understanding that the mere presence of organisms in the wound is less important than the level of bacterial growth.[2] The concept that the numerical level of bacteria was of clinical importance was suggested by Hepburn during World War I.[3] Attempts at decreasing the number of bacteria in wounds has led to a myriad of techniques, agents, and dressings being used. As new attempts were being tried, it was important to be aware of Thomas Sydenham's admonishment of *Primum non nocere*: above all, do no harm.[4]

Although Ignaz Philipp Semmelweis had demonstrated the effectiveness of chlorinated lime in preventing puerperal sepsis as early as 1861,[5] it was not until Henry Dakin, an English chemist, found that sodium hypochlorite (NaOCl) at a concentration of 0.5% was as effective as carbolic acid at killing bacteria, but less toxic to wound tissue than chloride compounds commonly used for wounds. Teamed with the French Army surgeon, Alexis Carrel, they found NaOCl at a concentration of 0.5% decreased fatal wound sepsis following combat wounds during World War I.[6] He had Carrel continually flood the wound site with the solution through rubber catheters inserted into the wound dressings, which became known as Dakin's solution. The problem was that it has a high pH, and when neutralized, it became ineffective. In addition, it was extremely unstable, which was the reason for the constant need for repeated irrigations.[7] Half-strength Dakin's solution (0.25%) became more popular to decrease the injurious effects to normal tissue.

In 1991, Heggers et al[8] demonstrated that a much more dilute solution of NaOCl could satisfactorily kill bacteria in wound tissue and not injure normal cells. This work with 0.025% NaOCl was the recipient of the Robert Lindberg Award of the American Burn Association.[9] Most clinicians who say they use and prefer Dakin's solution today actually are using the 0.025% NaOCl — not Dakin's solution. The problem is it still has a pH of 10 to 11 and is very unstable, becoming salt and water within minutes after application. Hidalgo et al[6] demonstrated that NaOCl in levels as low as 0.00005% are cytotoxic to fibroblasts.

Hypochlorous acid (HOCl), discovered in 1832, is a more stable compound than NaOCl and still has the antibacterial effects previously demonstrated for NaOCl.[6] The question, however, was whether the in vitro antibacterial effects transferred to tissue levels of bacteria in the in vivo wound situation. Robson et al[9] demonstrated that HOCl decreased the tissue level of bacteria in granulating chronic wounds while simultaneously allowing wound healing to proceed without any cytotoxicity. There are several hypochlorous products available and each has different pH levels and stabilities. Vashe is the most stable, has the highest concentra-

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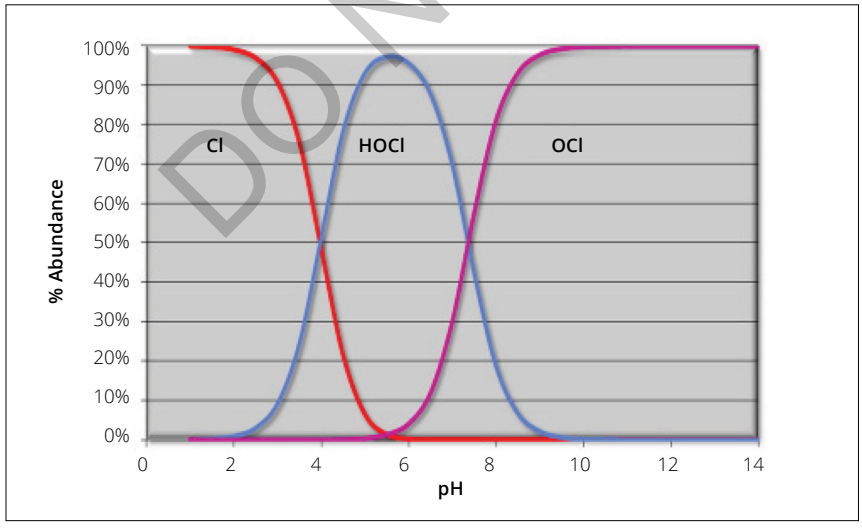

Figure 1. Distribution of chlorine species as a function of pH. The highest concentration of hypochlorous acid (HOCl) is approximately pH 5.5, the pH of Vashe Wound Solution.[9]

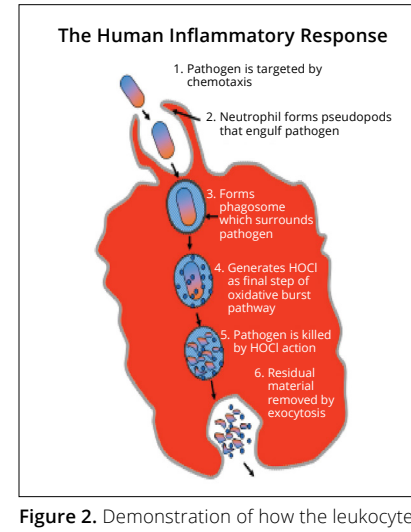

Figure 2. Demonstration of how the leukocyte generates hypochlorous acid (HOCl) to kill invading pathogens.[9]

tion of HOCl, and is at the pH of normal skin (**Figure 1**).

Hypochlorous acid has been shown to be bactericidal, fungicidal, and virucidal. To date, no resistance has been reported to Vashe. As a major defender against invading pathogenic microbes, HOCl destroys bacteria, fungi and their spores, and viruses within the human body as part of the innate inflammatory response (**Figure 2**). Recently, Dr. Robson worked with Dr. Bassiri of the University of California-Davis (Davis, CA) to investigate the killing mode(s) of action by Vashe. They determined Vashe kills bacteria by disruption and destruction of the bacterial cell wall and inhibition of DNA synthesis. These findings are important for several reasons. For instance, bacteria cannot survive without their wall. The barrier protects the inner workings of the organism, allows only certain materials to enter or exit the cell, and gives the bacteria its size and shape. Also, it is the structure that determines if it is a rod or a cocci, or if it is Gram positive or Gram negative, which are major factors in characterizing the organism. By inhibiting DNA synthesis, the bacteria are not able to reproduce, therefore, they will eventually die without making daughter cells to sustain the colony or the infection. Most importantly, when these 2 killing mechanisms act simultaneously, it results in nearly instant death for the bacteria. Some organisms can, with some success, overcome one mechanism of action against them, but when multiple mechanisms are present, as with Vashe Wound Solution, it is very difficult, if not impossible, to develop resistance.

There are other products that contain HOCl; however, none is in the pH range of Vashe. As seen in **Figure 1**, the pH range of Vashe dictates that it has the purest percentage of HOCl. Other products are more in the acidic range and will have chlorine species or in the basic range and have a percentage of NaOCl in their formulation.[10] As will be seen in the subsequent section, HOCl (specifically Vashe Wound Solution) has the additional advantage of being noncytotoxic.

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Hypochlorous Acid is a Noncytotoxic Alternative to Dakin's Solution for Wound Bed Preparation

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Wounds, particularly chronic and complex wounds, create a significant financial burden and negatively impact patient quality of life.[1] Chronic wounds are prevalent in the US population, and the cost to the health care system is staggering with the annual direct treatment costs of chronic wounds alone estimated at $40 to 60 billion.[2,3] Due to an aging population and an increasing prevalence of comorbid conditions (eg, diabetes), the burden of chronic wounds is growing. Acute wounds remain significant not only in the military setting but also in emergency trauma care. In addition, the need for postoperative wound care is on the rise.[4] According to the National Center for Health Statistics, an estimated 48 million inpatient surgical procedures were performed in 2009. The field of wound care has experienced tremendous growth and

development in response to the unmet needs of challenging wounds. In the past 2 decades a myriad of treatments, techniques, and technologies have emerged with variable results.[3,5]

Despite many promising advances, the foundation of effective wound care remains debridement, irrigation, and cleaning. Wound healing requires proper wound bed preparation.[6] The removal of dead tissue and application of cleansing fluid reduces bioburden and disrupts biofilms.[7] In the absence of intact skin, the contamination and colonization of wounds by bacteria is expected. Wound cleansers are utilized in part to reduce the risk of progression from bacterial colonization to invasive wound infection.[8] However, many commonly used wound cleansers have local cytotoxic effects that may impede wound healing. In this chapter, Dakin's solution is compared with the HOCl formulation of Vashe Wound Solution on bacterial killing, biofilm reduction, wound healing, and cytotoxicity.

Emergence of Dakin's Solution

The application of Dakin's solution to wounds was first described in 1915.[9] It is a dilute, buffered solution of (0.5%) NaOCl, or household bleach. Henry Dakin, a biochemist, developed the solution as an antiseptic, which dissociates to HOCl and alkali in water; the former, a potent bactericidal agent, and the latter, the caustic culprit of many of its irritating effects.[9] Working with Dakin, a surgeon named Alexis Carrel implemented Dakin's solution in the treatment of difficult wounds in field hospitals during World War I. His method involved the continual infusion of solution into prepared wound beds through networks of fenestrated rubber tubes.[10,11] The success of Dakin's solution has withstood the test of time, as it is still used as an antiseptic treatment in a variety of complex wounds today.[12,13] However, Henry Dakin himself noted several shortcomings of his solution in his original paper,[9] namely a short period of therapeutic action and the potential to irritate healthy tissues.

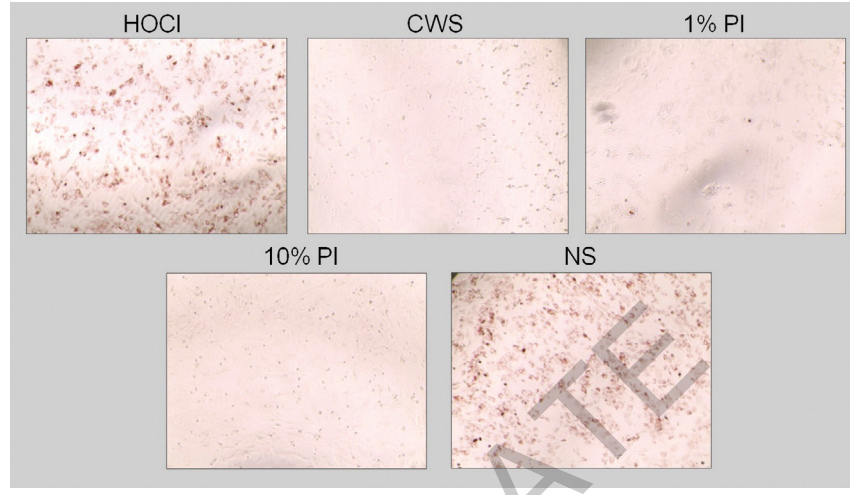

Figure 1. In vitro cytotoxicity. Representative images of fibroblasts from the neutral red dye assay; the presence of many stained cells indicates that the treatments to those cells were minimally cytotoxic. HOCl: hypochlorous acid; VWS: Vashe Wound Solution; CWS: chlorhexidine wound solution; PI: povidone-iodine; NS: normal saline

History of HOCl

Hypochlorous acid has long been recognized as an effective antiseptic. Initially discovered in 1788 by the French chemist Berthollet, various formulations of HOCl solutions have been developed. James Lorrain Smith, a Professor from Edinburgh, developed "Eusol" during World War I; however, it was outperformed and overshadowed by Dakin's solution due to its exceedingly unstable and relatively impure chemistry.[14,15] Hypochlorous acid is the final product of the respiratory burst in neutrophils and has an important role in innate immunity. Until recently, the development of HOCl as an antiseptic solution likely was limited by challenges in maintaining storage stability.[16] The introduction of a commercially available solution of HOCl with long-term stability has reinvigorated the use of HOCl as an antiseptic solution for wound care.[17]

More Than Antisepsis: Cytotoxicity is Important to Wound Closure

When applying a cleansing agent to a wound, clinicians should consider the cleanser's bactericidal ability against its cytotoxic effects on healing tissue.[18] Cleansing wounds to remove residual debris and prepare the wound bed through reduction of bacterial load is a basic component of wound management.[6] There is significant variability in the cytotoxicity of available cleansers, and optimal initial wound management should not interfere with subsequent healing.[19]

Dakin's Solution is Cytotoxic

Barsoumian et al[20] evaluated the cytotoxicity of Dakin's solution in vitro using time-kill studies of cultured human cell lines. They identified a dose-dependent decrease in toxicity to fibroblasts, keratinocytes, and osteoblasts, with concentrations less than or equal to 0.00025% being safe, some toxicity at 0.0025%, and near complete toxicity at higher concentrations. They did not appreciate a difference in toxicity over time.[20] Similarly, Wilson et al[21] exposed cultured infant fibroblast and keratinocyte cells to serial dilutions of Dakin's solution and used an MTS cell proliferation/viability assay to identify the dilution required to generate 85% viability when compared with a control. A solution of 0.0025% and 2.5 x 10^{-7}% Dakin's met these criteria for fibroblasts and keratinocytes, respectively.[21] Dakin's solution (0.5% NaOCl) is marketed in full, half (0.25%), quarter (0.125%), and 1/40 (0.0125%) strength formulations.[22] Results from the aforementioned

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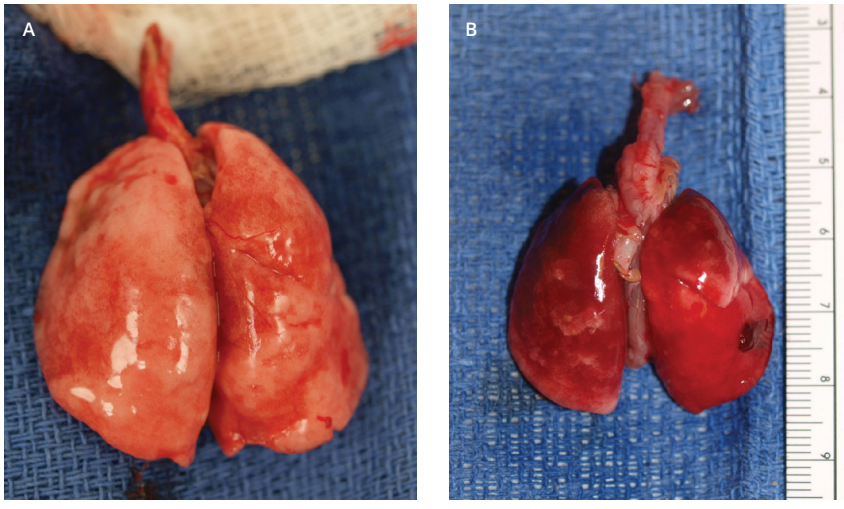

Figure 2. Gross examination of organs after lavage. Gross specimens of lungs treated with (A) Vashe Wound Solution (VWS) and (B) Dakin's solution. The Dakin's-treated lungs show increased fibrosis and hemorrhage compared with VWS.

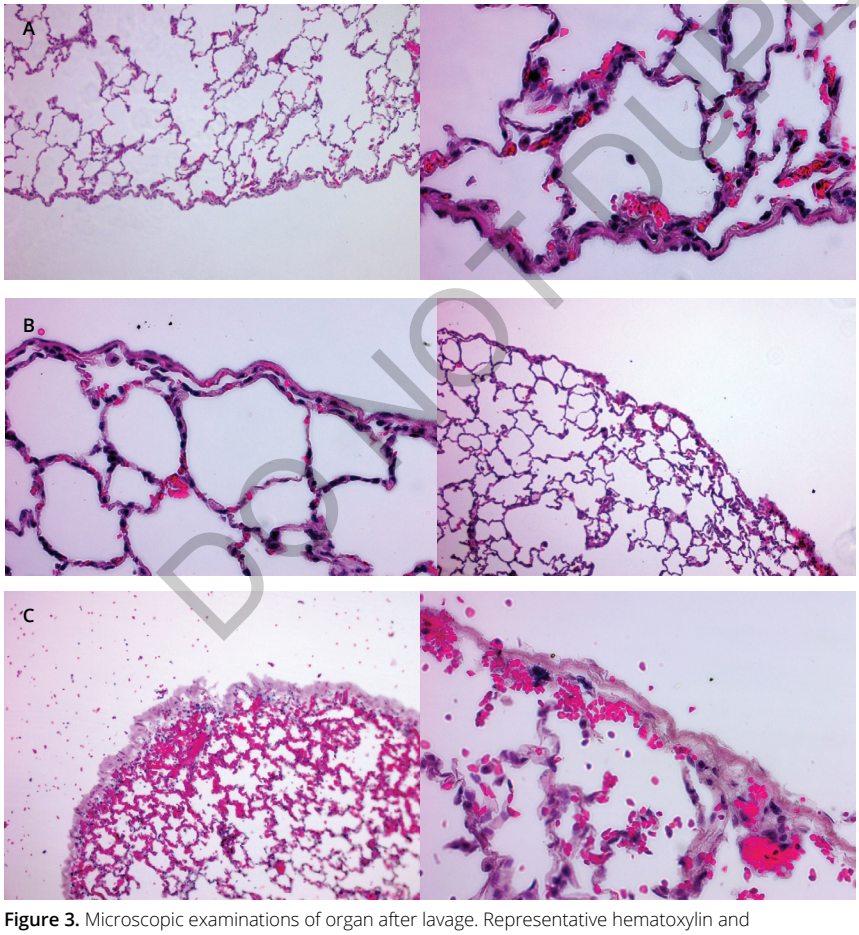

Figure 3. Microscopic examinations of organ after lavage. Representative hematoxylin and eosin-stained images of lung tissue treated with (A) normal saline, (B) Vashe Wound Solution, and (C) Dakin's solution. Increased fibrosis and hemorrhage are appreciated in the Dakin's-treated tissue.

studies[20-22] suggest all of these concentrations are cytotoxic to healing cells in vitro.

HOCl Preparations Lack Mammalian Cytotoxic

Day et al[23] assessed the cytotoxicity of HOCl solution in vitro using a neutral red dye assay with primary human fibroblasts. They found 71% of cells survived a 3-minute incubation with the undiluted HOCl solution, which was superior to chlorhexidine, and povidone-iodine solutions (**Figure 1**).[23] Further investigations from this lab compared the cytotoxicity of HOCl solution with Dakin's solution in vivo. Briefly, rodents underwent thoracotomy, laparotomy, or laminectomy and intracavitary lavage with normal saline, HOCl, or Dakin's solution. There were 2 lavages for 2 minutes each, at which point the fluid was aspirated and the incisions closed. On postoperative day 5, the animals were euthanized and organs examined grossly (**Figure 2**) and histologically (**Figure 3**). There was no difference between the normal saline and HOCl groups, while the Dakin's group showed evidence of fibrosis and hemorrhage, as well as increased evidence of apoptosis on immunohistochemistry.[23]

HOCl Preparations Reduce Bioburden and Biofilms

The microbicidal efficacy of antiseptic wound cleansers is usually proportional to negative effects on healing tissue.[24] Ultimately, wound cleansers are not selective in their mechanism of action, and the ideal agent would be maximally bactericidal with minimal toxicity to healing tissue.[6] While the presence of bacteria in a wound alone does not impede healing, biofilms form quickly, protect and propagate micro-organisms, and stimulate a chronic inflammatory response that delays wound healing and creates opportunity for invasive local infection.[7]

The antimicrobial properties of hypochlorites have long been established. Clinical experience since the invention of Dakin's solution has proven that such antiseptic solutions are effective in wound management. Heggers et al[25] sought to

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identify an *efficacious zone* for Dakin's solution, recognizing its excellent antibacterial capabilities and responding to concerns about cytotoxicity. Ten species of Gram-negative and Gram-positive bacteria were exposed to 3 different concentrations of Dakin's solutions, and 0.025% Dakin's solution proved bactericidal to all strains at 30 minutes.[25] Lindfors[26] evaluated the clinical efficacy of 0.05% NaOCl solution in reducing wound bioburden. There were 11 patients and 18 wounds cleansed with normal saline, or Dakin's solution, in addition to standard of care. The wounds were swabbed and cultured to quantify aerobic and anaerobic bacterial bioburden. There were 9 wounds in each group, and bioburden was reduced by 1 to 4 $\log_{10}$ colony forming units (CFUs) in all wounds treated with NaOCl at 2 weeks. One-third of the wounds treated with normal saline showed bioburden reduction while more than half showed an increase.[26]

Day et al[23] evaluated the bactericidal effect of Vashe Wound Solution on biofilm in vitro. Methicillin-resistant *Staphylococcus aureus* (MRSA; ATCC 43300) and *Pseudomonas aeruginosa* (ATCC 10145) were used to grow biofilms. They were exposed to Vashe Wound Solution, chlorhexidine solution, 1% or 10% povidone-iodine solution, or normal saline. At 3 minutes of treatment, Vashe and chlorhexidine showed greater than 99% reduction in CFUs compared with normal saline, while 10% povidone-iodine eliminated nearly all viable cells. Similar, but more robust results were appreciated with *P aeruginosa* biofilms at 3 minutes.[23] Robson et al[27] evaluated the bactericidal efficacy of HOCl solution at various pH levels and applications in vivo. They utilized a murine model of a chronic granulating wound and took wound biopsies at several timepoints, expressing mean bacterial counts in each treatment group as CFU/g of tissue. During the study, they optimized the dosing regimen for HOCl solution based on preliminary data of bacterial load reduction. Their data showed a 15-minute application, followed by atraumatic wiping, and a second dressing that remained in place until the next daily dressing change was most effective. There was a reduction in bacterial bioburden by 5 to 6 $\log_{10}$ CFU/g in all groups exposed to this treatment.[27]

Duarte et al[12] present a contemporary case report of the use of Dakin's solution as an adjunct for the salvage of a severely infected diabetic foot. After emergent incision and drainage, negative pressure wound therapy was ineffective, and marked improvement was appreciated with the instillation of Dakin's solution. The wound was fully granulated at 6 weeks.[12] Lindfors[26] observed wound healing rates among patients treated with Dakin's solution in addition to standard of care compared with normal saline. Statistical significance was limited by study size; however, among 11 patients (18 wounds), 22% of wounds treated with Dakin's decreased in size over 2 weeks versus 11% treated with normal saline. No wound treated with Dakin's increased in size compared with 56% of wound treated with saline. No tissue damage or toxicity was observed in either group.[26]

Odom et al[28] utilized Vashe Wound Solution for wound bed preparation on 4 patients with chronic, contaminated wounds harboring MRSA, vancomycin-resistant *Enterococcus*, and *Pseudomonas* prior to treatment. At the time of skin grafting, wound cultures were negative and the appearance of the wounds had improved. At 2 weeks, all patients had healed skin grafts without evidence of infection. Vashe Wound Solution successfully was applied directly to healing skin grafts given its noncytotoxicity and physiologic pH.[28] Niezgoda et al[17] found Vashe Wound Solution was an effective adjunct to standard of care for patients with large, chronic venous leg ulcers. Their observational study reported 10%, 55%, and 79% of wounds showed complete reepithelialization at 30, 60, and 90 days, respectively. Interestingly, pain and odor (present in 77% and 67% of patients, respectively), prior to treatment, was zero at the conclusion of the study, with gradual reduction in scores after initiation of Vashe treatment.[17] The physiologic pH of Vashe Wound Solution allows it to be safely used around the eyes, mouth, and mucous membranes and is likely responsible for the soothing effect that patients often endorse on its application.[29]

Conclusions

Chronic and complex wounds severely impact quality of life for patients, and treatment places financial stress on individuals and institutions. Despite innovation in the field of wound care, debridement, irrigation, and cleansing remain the foundation of wound management. There are a variety of antiseptic wound cleansers in the clinician's armamentarium. Dakin's solution is an effective bactericidal agent with a history of success as a wound sterilizer, especially as a salvage for contaminated, complex wounds. However, as the understanding of wound care has evolved, its cytotoxic effects have been placed under scrutiny. The optimal wound cleanser is bactericidal and noncytotoxic to healing tissues, and Vashe Wound Solution has been proven to possess these qualities; thus, it should be considered for most wound cleansing applications.

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Indications for the Use of Hypochlorous Acid in the Burn Patient

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Burn injury is a common cause of injury in most parts of the world.[1] Burn injuries are susceptible to infection for several reasons, including loss of the protective skin layer, immunosuppression that accompanies burn injury, and the necessity for operative management of the burn wound.[2–4] Thus, topical antimicrobial protection is the standard of care for burn excision and skin grafting.

Commonly used topical antimicrobial agents include silver sulfadiazine cream, mafenide lotion, povidone-iodine solution and mafenide acetate 5% solution, Dakin's solution, and other antimicrobial creams, lotions, ointments, and solutions. Many of these topical agents have adverse effects on healing. Both povidone-iodine and mafenide solution are known to be toxic to mammalian cells and, thus, might have a detrimental influence on wound healing.[5,6] In addition, absorption of iodine may lead to systemic toxicity,[7,8] whereas mafenide may be painful and lead to metabolic acidosis through inhibition of carbonic anhydrase.[9] Allergic reactions to both materials have been described as well.

Thus, while topical antimicrobial therapy is necessary to protect against infection, agents commonly used often have adverse effects,

therefore, are not ideal candidates for topical antimicrobial therapy. Hypochlorous acid (HOCl) is a topical antimicrobial with many desirable characteristics. It is produced in vivo by neutrophils as part of the respiratory burst pathway.[10] This pathway plays a crucial role in intracellular killing of microorganisms by leucocytes.[11-13] Hypochlorous acid has been shown to rapidly kill Gram-positive and Gram-negative microorganisms, including methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococcus*.[14,15] Microbial resistance to HOCl has not occurred; it is thought to have antimicrobial properties via a number of different mechanisms at the plasma membrane location.[16-18] In addition, HOCl has no toxicity to human cells when used in a clinically effective dosage.[19-26] Data have been generated for HOCl for various indications of burn treatment.

FDA-approved Indications

The US Food and Drug Administration (FDA) has granted 510(k) approval to Vashe Wound Solution as a device. Its approval states, "Under the supervision of healthcare professionals, Vashe Wound Solution is intended for cleansing, irrigating, moistening, debridement, and removal of foreign material including microorganisms and debris from exudating and/or dirty wounds, acute and chronic dermal lesions, such as Stage I-IV pressure ulcers, stasis ulcers, diabetic foot ulcers, ingrown toe nails, grafted and donor sites; and exit sites. It also is intended for moistening and lubricating absorbent wound dressings." From the FDA approval, it is clear that it has many indications that apply to the burn patient.

Indications for Postoperative Skin Graft Dressings

Recently, Foster et al[27] reported the results of a randomized trial comparing HOCl with 5% Sulfamylon (Mylan, Canonsburg, PA) solution as a topical therapy following skin grafting. Patients with burns requiring skin grafting were randomized to HOCl or 5% Sulfamylon solution as topical dressings postoperatively. Inclusion criteria included thermal injury 20% or more total body surface area requiring excision and autografting, and aged 18 years or older. Exclusion criteria included pregnant women, chlorine sensitivity, and electrical/chemical/cold injuries. The following outcomes were assessed: patient demographics, graft viability, infection, pain score, narcotic usage, adverse events, and cost.[27]

Treatment groups were demographically equivalent. There were no differences in adverse or serious adverse events between the 2 groups. Graft viability and infection were equivalent between the 2 groups. In addition, pain scores and narcotic usage were similar. Hypochlorous acid was significantly less expensive than 5% Sulfamylon solution, thus more cost effective. The study concluded that HOCl demonstrated equivalent efficacy and safety compared with 5% Sulfamylon solution when used as a postoperative topical dressing for skin grafts. Although the study was small, it demonstrated that HOCl solution is indicated for treatment of skin grafts.[27]

Indications for Decolonization in a Burn Intensive Care Unit

Infections are a leading cause of morbidity and mortality in burn patients. Patients colonized with MRSA are at higher risk of developing invasive infection. Gray et al[28] reported a study of decolonizing burn patients with HOCl bathing and nasal mupirocin. Global MRSA infection rates per 1000 patient days decreased from 7.23 pre-intervention to 2.37 when the universal decolonization protocol was used. They concluded that the protocol using HOCl bed baths as part of their decolonization protocol led to a significant decrease in MRSA infections.[28]

Conclusions

From the aforementioned studies, it is clear that there are data-supported indications for use of a HOCl product such as Vashe Wound Solution in the treatment of burn patients.

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Hypochlorous Acid Solution as Burn Wound Antimicrobial: Implications of Burn Depth and Area

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The assessment of burn depth relies almost entirely on the clinician's bedside visual exam of the wounds. In general, superficial first-degree burns appear erythematous and are without blisters. Capillary blanching is rapid. Partial-thickness or second-degree burns feature blisters, blanch readily, and are moist and sensate on exam. Full-thickness or third-degree burns have a leathery appearance and are dry, inelastic, and insensate. Blanching is absent in wounds in the setting of capillary bed injury and thrombosis.[1]

Several factors confound the accurate assessment of wound depth by visual exam. The previously described wound characteristics, particularly those of deeper burns, develop over time and may not be readily apparent upon presentation. Devitalized tissue may take much longer than 24 hours to reveal its color differentiation, lack of capillary refill, and loss of elasticity. What appears to be a partial-thickness injury on presentation may become visually apparent as a very deep dermal or full-thickness injury only after significant time passes. The anatomic region affected by the burn injury also affects the ease of burn depth assessment. Skin regions with a thick dermal component, such as the posterior torso, are more likely to have intact deep dermal structures after thermal injury compared with regions with a thin dermal component, such as the dorsum of the hand.

Adjuncts to burn depth assessment, including histology and various techniques of measuring tissue perfusion have not gained traction due to issues of logistics and reproducibility. As stated above, full-thickness burns are dry to the touch and feature an inelastic eschar. These are characteristics of a denatured extracellular protein matrix. This collection of devitalized tissue provides a physical barrier to the influx of many agents typically used to cleanse wounds. While an effective barrier to external wound cleansing agents, the eschar is easily infiltrated by microbes, such that it serves as an important depot of infectious potential.[2]

As the percent of burn wound total body surface area (TBSA) increases, uniformity of burn depth decreases and the accuracy of burn depth estimation by visual exam suffers. The thermal energy exposure needed to inflict a 40% or more TBSA wound makes these injuries unlikely to have solely partial-thickness depth in all areas. The "indeterminant depth" burn is one which cannot be accurately staged

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at the time of presentation. Subsequent observation over days to weeks may reveal deep dermal (reticular dermis) damage masked on initial exam. Surgical tangential excision itself is notoriously imprecise in removing only necrotic skin and leaving viable dermis behind.[3] Other challenges of the large % TBSA burn include potential delays in escharectomy secondary to patient physiology and re-accumulation of proteinaceous wound debris necessitating serial debridement procedures prior to definitive closure. As hospital length of stay increases with increasing burn size, the rates of graft failure, conversion of acute to chronic wounds, and bacterial resistance increase.[4]

There are several desirable characteristics in a topical antimicrobial used in burn care. These include eschar penetration, a broad spectrum of activity across normal skin flora, Gram-negative bacteria, and opportunistic organisms such as fungi; stability among proteinaceous wound debris; and no toxicity against human fibroblasts. Reproducible eschar penetration is noted only with formulations of mafenide acetate, a compound in widespread use over the last 6 decades for burn care and responsible for reductions in rates of deadly burn wound sepsis. In spite of effective eschar infiltration with this antimicrobial, early proponents of this compound also cautioned that topical antibiosis was only one arm of a balanced regimen that included adequate observation and surgical excision.[5] The cytotoxic nature of high concentrations of this compound continues to fuel the search for alternate treatments. In this setting, although hypochlorous acid (HOCl) solutions offer in vitro bactericidal activity with little or no reported cytotoxicity, these solutions do not efficiently transit the intact unexcised eschar of a full-thickness burn.[6]

Many microbes create biofilms as a primary counter to antibiosis. Among these, methicillin-resistant *Staphylococcus aureus* and *Pseudomonas aeruginosa* have been studied in concert with HOCl activity against their biofilms. Biofilm disruption by HOCl was documented by Day et al,[7] comparing this activity versus normal saline controls. This disruption, which occurs along with lavage and other mechanical means, may be extrapolated to the eschar problem. The heavily proteinaceous environment of the eschar provides a mechanical barrier to antisepsis and therefore must be mechanically disrupted. Of note, even normal saline showed some reduction of bioburden in these biofilms, but without the addition of an antimicrobial, significant numbers of viable bacteria were identified after lavage. This informs an important lesson of full-thickness burn eschar management; multimodal therapy combining physical debridement, lavage, and antimicrobial application is the key to reducing viable bioburden.

Treatment failures of HOCl solutions, and indeed other antimicrobials, must be examined in the context of the burn depth and wound care procedure utilized. Failure to provide debridement of eschar as part of a balanced strategy of full-thickness burn management is a recipe for poor results. Although early tangential excision of full-thickness eschar is pursued at most centers, in certain situations clinicians may find themselves faced with treating full-thickness injuries with topical strategies as a bridge to surgical management. Current recommendations for the use of HOCl solutions for burn care must include:

1) A primary antimicrobial solution in acute, unexcised burn wounds with clear partial-thickness depth on visual exam and minimal eschar or debris;
2) A wound cleansing agent combined with mechanical debridement and lavage;
3) A post-excision antimicrobial solution;
4) Avoiding unexcised full-thickness burn wounds as sole antimicrobial;
5) Avoiding unexcised large surface area "indeterminate depth" burn wounds as sole antimicrobial; and
6) Avoiding the use of HOCl with chlorhexidine gluconate, because it will inactivate HOCl.[8]

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Vashe Wound Solution is Effective to Control *Pseudomonas aeruginosa* Wound Colonization

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Pseudomonas aeruginosa is an encapsulated Gram-negative rod that normally resides in soil and water. Due to its high antibiotic resistance, it is capable of surviving in various natural and synthetic environments.[1-3] It is a facultative anaerobe and thrives in conditions with little or no oxygen.[4] It is a leading cause of nosocomial infections and contributes to high morbidity and mortality, particularly in immunocompromised patients.[1,2] Because of its emerging antibiotic resistance, infections caused by *P aeruginosa* are becoming more challenging to treat.[5]

P aeruginosa causes airway infections in patients with pre-existing lung diseases such as cystic fibrosis.[6] Excess mucus production with limited oxygen diffusion in these disease processes provides the ideal environment for *P aeruginosa* to grow.[2] In immunocompromised patients, *P aeruginosa* infects the airway, urinary tract, and indwelling catheters.[1] In patients with diabetes and burns, damaged tissue, damp wound environment, and altered immune response allow *P aeruginosa* to cause severe wound infections.[5-7] Pyoverdine, a green metabolite produced by *P aeruginosa* gives the wound its characteristic green color.[3] *P aeruginosa* causes a superficial wound infection, which may progress to invasive infection and sepsis, if left untreated. Research has shown 60% of chronic wounds contain *P aeruginosa*.[8] Treatment of *P aeruginosa* is difficult due to the emergence of multidrug-resistant strains.[9]

Pathogenicity

The pathogenesis of *P aeruginosa* involves quorum sensing, a process that involves activation of signaling cascade to eventually produce virulence factors and biofilms. The first step in *P aeruginosa* infection is attachment and colonization of the host. A protease produced by *P aeruginosa* degrades the fibronectin cell wall of human host exposing pili receptors.[3] The type IV pili bind to receptors on epithelial cells of the upper respiratory tract or cutaneous wounds. Type IV pili also helps with adherence and colony formation, which facilitates biofilm formation. Bacteria secrete extracellular polymers that construct the layer of film around them.[3] Biofilms contribute to antibiotic resistance by acting as a barrier to antibiotic penetrance.[3,8]

P aeruginosa contains a protein called type III secretion system, which injects effector toxin proteins in host cells, which inhibit cell defenses and allow *P aeruginosa* to survive. *P aeruginosa* contains 4 effector toxins named exoenzymes S, T, U, and Y. Exoenzymes S, T, and Y prevent phagocytosis by the host cells. ExoU has phospholipase A2 activity and can lyse cell membranes by cleaving phospholipid residues. Furthermore, ExoU contributes to inflammation by activating the cyclooxygenase pathway.[3]

P aeruginosa also has several virulence factors of which exotoxin A is most toxic.[10] It binds to the alpha-2 macroglobulin receptor on the fibroblasts, enters host cell, and robosylates elongation factor 2 leading to host cell death. Furthermore, studies have shown exotoxin A is linked with iron and glucose metabolism. Pyoverdine is involved in a signaling pathway that upregulates exotoxin expression. Thus, exotoxin A plays an important role in the evasion of host immune response of *P aeruginosa*.[11]

Biofilm and antibiotic resistance

P aeruginosa intrinsically has the ability to develop antibiotic resistance. Inadequate antibiotic therapy and inappropriate use of antibiotics also has contributed to antibiotic resistance.[3]

Hypochlorous acid

Hypochlorous acid (HOCl) is formed in leukocytes during oxidative burst; a process in which oxygen is catalyzed, in the presence of nicotinamide adenine dinucleotide phosphate, to oxygen radicals that function to kill microbes. Oxygen radicals subsequently form hydrogen peroxide, which is converted to HOCl by the action of myeloperoxidase.[12]

Vashe Wound Solution is essentially pure HOCl and an effective microbicidal solution.[12] At clinically safe doses it is not toxic to human cells. It has been shown to kill a variety of bacteria including *Staphylococcus aureus, Bacillus* spp, and *P aeruginosa*.[12] It also has fibrinolytic properties and reduces wound exudate in venous leg ulcers. Vashe has been shown to be superior to normal saline for wound irrigation and is effective in reducing pain experienced by patients with chronic leg ulcers.[13]

Despite the evidence, concern remains over the effectiveness of Vashe Wound Solution against different strains of *P aeruginosa*. Furthermore, Vashe can degrade hemoglobin and result in formation of verdoglobin (also called verdohemoglobin or ferri-biliverdin), a molecule formed when heme group is attached to biliverdin.[14] Verdoglobin is green in color and results in the green color of the wound, which can be mistaken for the pyoverdine produced by *P aeruginosa*. Previous work in this laboratory has demonstrated

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that Vashe is less cytotoxic to cells and equally comparable to chlorhexidine solution at reducing *P aeruginosa* biofilms in isolate ATCC 10145.[15] The purpose of this study is to examine the effects of Vashe on 5 different *P aeruginosa* isolates.

Methods

Bacterial strains

Five strains were selected from the Centers for Disease Control and Prevention (CDC) *P aeruginosa* panel. Strains 246, 249, and 250 are mostly antibiotic resistant, and strains 262 and 263 are mostly antibiotic sensitive. These were grown according to the manufacturer's instructions and stored at -80°C prior to the start of the study.

Biofilm growth

P aeruginosa isolates were incubated overnight aerobically at 37°C in tryptic soy broth (TSB; Sigma Aldrich, St. Louis, MO) and nutrient broth (NB; BD, Sparks, MD). After 24 hours, these were incubated aerobically at 37°C, in presence of fresh TSB and NB, on precision shaking water bath (ThermoFisher, Frederick, MD) at 90 rotations per minute to achieve 10^8 colony forming units (CFUs). These cultures then were plated onto 35-mm diameter polystyrene plates (BD Falcon, Franklin Lakes, NJ), coated with type I collagen, and incubated at 37°C for 24 hours for biofilm formation.

Wound cleanser treatment

To compare effectiveness of various wound treatment solutions, isolates were treated with Vashe Wound Solution, 0.05% chlorhexidine wound solution (Irrimax Corporation, Gainesville, FL), 10% povidone-iodine (CareFusion, Vernon Hills, IL), mafenide acetate, or normal saline for 3 minutes.

CFU assay

After applying different wound treatment solutions, phosphate buffered saline (PBS) was used to wash the biofilms. Cell scrapers were used to capture the biofilms. Serial dilutions were performed using PBS, and *P aeruginosa* was plated on nutrient agar and allowed to grow aerobically for 24 hours at 37°C. After 24 hours, the average CFUs were calculated per milliliter for each strain. Student *t* test was used to calculate the difference between controls and treatment groups. GraphPad Prism 6.0 (GraphPad Software, La Jolla, CA) was used for statistical analysis; statistical significance was set at a *P* value of .05.

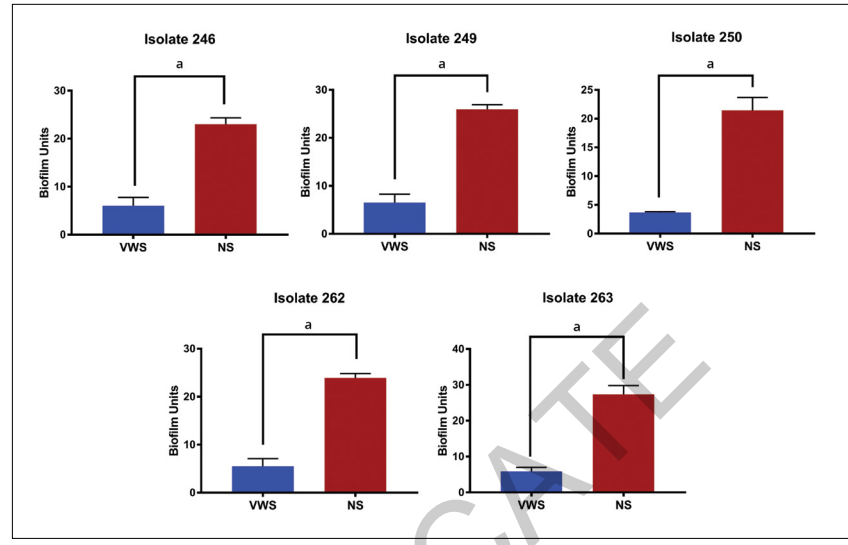

Figure 1. Amount of reduction in bacterial levels in *Pseudomonas aeruginosa* biofilms treated with 10% povidone-iodine, VWS, or NS for 10 minutes.
[a] *P*<.0001 vs. NS. Biofilms treated with 10% povidone iodine had too few colonies to count.
VWS: Vashe Wound Solution; NS: normal saline

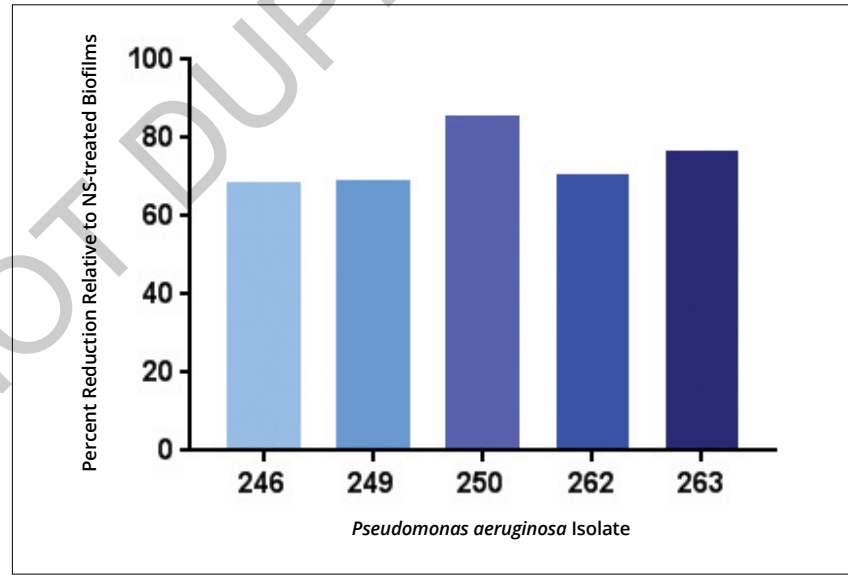

Figure 2. Comparison of the percent reduction in bacterial levels for *Pseudomonas aeruginosa* isolates.
NS: normal saline

Agar well diffusion assay

P aeruginosa isolate was grown and inoculated on Müeller Hinton agar plates and incubated at 37°C for 24 hours. A 6-mm punch biopsy was used to create a well near the center of

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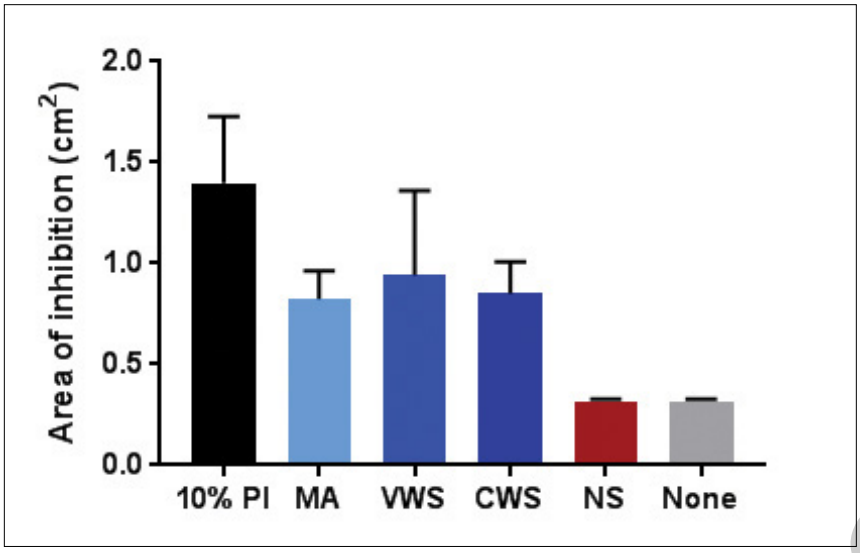

Figure 3. Comparison of areas of inhibition of *Pseudomonas aeruginosa* isolate 250 around wells containing 10% povidone-iodine (PI), mafenide acetate (MA), Vashe Wound Solution (VWS), chlorhexidine (CWS), normal saline (NS), or no treatment (none).

each plate and 100µL of one of the following treatment solutions was added to the wells: 10% povidone-iodine, 0.25% Dakin's solution, 0.05% chlorhexidine, Vashe Wound Solution, mafenide acetate, or normal saline. Each test was performed in duplicate and the control plate did not receive any treatment. Agar plates were incubated at 37°C for 24 hours and then ImageJ software (Version 1.52a; National Institutes of Health, Bethesda, MD) was used to quantify reduction in bacterial growth.

Results

Reduction in biofilm in 5 isolates

Quantitative bacterial cultures showed Vashe Wound Solution treatment of biofilms produced by isolates 246 (n = 6) or 249 (n = 6) resulted in a 69% reduction in biofilm compared with normal saline treatment (n = 4, n = 5, respectively) (**Figures 1, 2**). For isolate 250 (n = 6), 262 (n = 5), and 263 (n = 6), Vashe treatment reduced biofilms by 85%, 71%, and 77%, respectively, when compared with normal saline-treated biofilms (n = 6, n = 3, and n = 3, respectively). Reductions in bacterial numbers in Vashe groups compared with saline groups were statistically significant ($P < .001$). Also, 10% povidone-iodine eliminated bacterial biofilm viability for all isolates.

Agar well diffusion assay for isolate 250

For the agar well diffusion assay, the plates with Vashe Wound Solution in the wells produced a larger area of inhibition compared with the plates that had normal saline and those that did not receive any treatment, though the results were not significant (**Figure 3**). Vashe, chlorhexidine, and mafenide acetate produced similar areas of inhibition. In addition, 10% povidone-iodine created the largest area of inhibition.

Discussion

P aeruginosa is a Gram-negative bacteria responsible for life-threatening infections in immunocompromised patients, such as those with cystic fibrosis, diabetes, and burns.[1] Its various virulence factors, along with its ability to form biofilms, contribute to antibiotic resistance, making it a challenging pathogen to treat. Various wound treatment solutions have been used over time to treat *P aeruginosa* wound infections. However, none are perfect due to a high cellular toxicity. Vashe Wound Solution has previously been shown[12,13,15] to be a noncytotoxic and effective microbial solution. This study shows Vashe was superior to normal saline at reducing *P aeruginosa* biofilms in all 5 isolates.

Vashe Wound Solution proved to be as effective as chlorhexidine and mafenide acetate, and superior to normal saline at inhibiting bacterial growth. These results were not significant, likely due to the small sample size. The noncytotoxic profile of Vashe makes it a better choice for wound treatment. Although 10% povidone-iodine solution was most effective at bacterial growth inhibition and decreasing bacterial counts, it has been shown to be cytotoxic and impair wound healing,[15] making it a suboptimal choice for a wound treatment solution and more importantly for wound bed preparation. Furthermore, Vashe is effective at reducing biofilm production for 5 different *P aeruginosa* isolates compared with normal saline.

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Disruption of Biofilm With Vashe Wound Solution

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Wound healing is a complex process with many potential factors that can delay healing, including bacteria. There is increasing evidence that some bacteria within chronic wounds live within biofilm communities in which bacteria are protected from host defenses and develop resistance to systemic antibiotic treatment.[1] Bacteria in biofilm behave differently from planktonic bacteria of the same organism in terms of their response to antibiotic treatment and human immunity.[2,3] Biofilm is formed when a group of microorganisms stick to each other and become embedded within a self-produced matrix of extracellular polymeric substance composed of extracellular DNA, polysaccharides, and proteins.[3] The subject of wound biofilm is a complex concept that was first postulated in the 1990s. It was popularized at the University of Cardiff by Dr. Keith Harding and his associates to attempt to explain why a slime or scab occurring on chronic wounds could not be killed or eradicated by systemic or topical antimicrobials known to eliminate bacteria cultured from the wounds.[1] They coined the concept of wound biofilm and postulated that the bacteria were protected by a matrix consisting of proteins and polysaccharides produced by the bacteria. The concept was popularized by non-surgeons, because surgeons believed biofilms existed on the surface of wounds and could be easily removed physically by different methods of debridement.

However, since the bacteria in the surface biofilms are protected by the extracellular matrix, attempts to eliminate the biofilm with simple debridement, dressings, or antimicrobial agents such as antibiotics, chemicals, or silver products have been largely futile. It is much better to disrupt the biofilm physically, and then the unprotected surface bacteria can be eradicated easily. The disruption of biofilm requires physical manipulation, not chemical reactions, which was demonstrated by Wolcott[4] using a hydroconductive dressing (Drawtex; Urgo Medical, Fort Worth, TX). Because this dressing has no antibacterial properties, Wolcott[4] was able to totally destroy wound biofilms while quantitative evaluations of the bacterial population demonstrated no decrease in the bacterial population. The author postulated that the physical action of the hydroconductive dressing drew out exudate from the wound, and in that exudate, there were nutrients necessary to propagate the biofilm. Therefore, this was a physical disruption of the biofilm.

Various wound cleansers have shown the ability to physically disrupt biofilm.[5] Hypochlorous acid (HOCl; Vashe Wound Solution) is an example of a wound cleanser. How does it physically disrupt biofilm? Polysaccharides are long chains of simple sugars (monosaccharides) that can be broken down easily by most liquids—as simple as water. Soaking a biofilm with water or Vashe physically will break down the polysaccharide portion of the protective biofilm matrix. This is the process of hydrolysis, which is defined as the splitting of a chemical compound into 2 or more compounds by reacting with water. Another way polysaccharides can be broken down is by chemical enzymatic reaction, but this is not postulated to be the action of Vashe.

The second part of the protective matrix of biofilm is protein. There are 2 basic ways protein is broken down. The first is by enzymatic

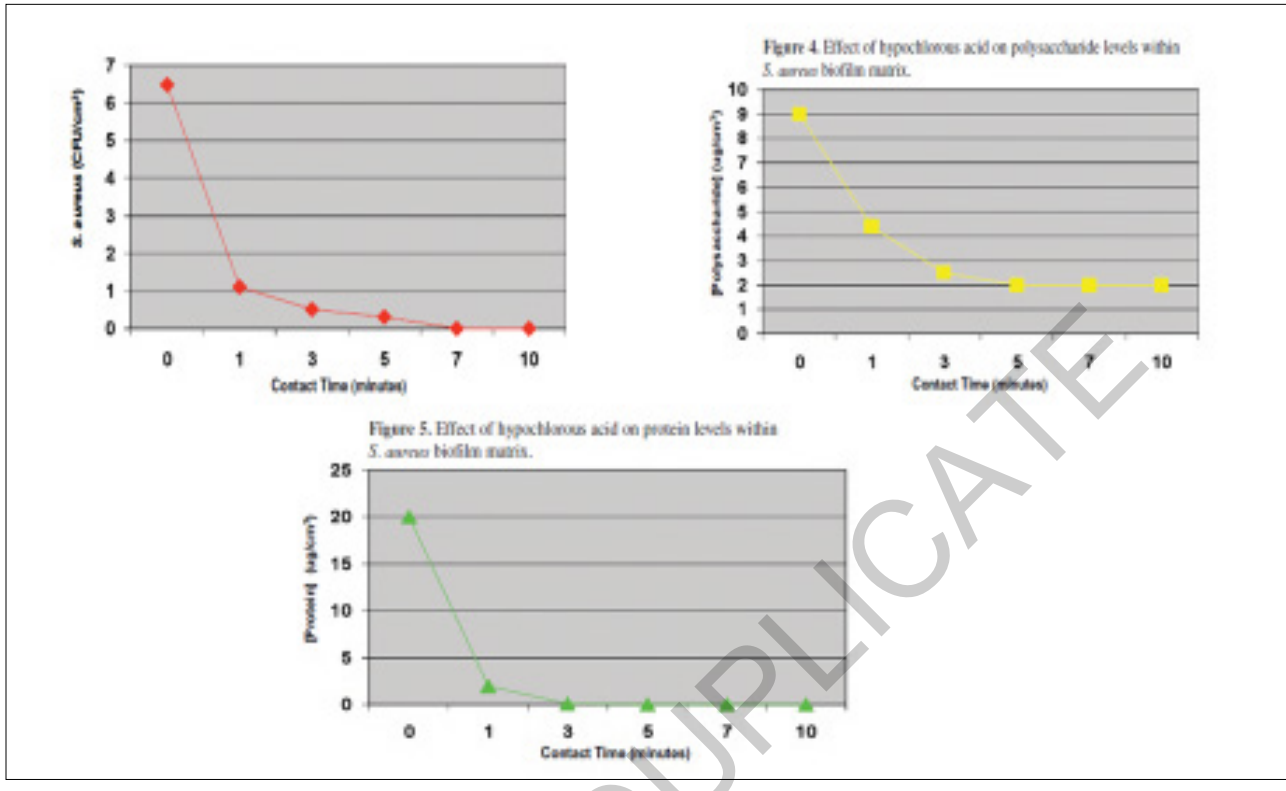

Figure. Exposure of in vitro biofilm to hypochlorous acid over a 10-minute time period showing reduction in *Staphylococcus aureus*, polysaccharide, and protein.

proteolysis as occurs with food digestion. The second is a physical mechanism of nonenzymatic proteolysis. Proteins, as macromolecules, can be damaged physically by free radicals and products of oxygen metabolism such as HOCl.

Since both the polysaccharides and proteins of the protective matrix can be disrupted physically, biofilms can be disrupted by other means than just chemical. Once this occurs, the unprotected bacteria, lying on the surface of the wound, are susceptible to almost any substance known to damage or kill bacteria in vitro or in vivo. Studies of biofilm in vitro and in vivo treated with Vashe demonstrate that polysaccharides and proteins are decreased, because they have been broken down physically[6,7] (**Figure**). The now exposed bacteria are decreased, as if they are in an in vitro test tube.[8] It is not suggested that Vashe is killing or eradicating bacteria in the wound, but rather that the extracellular protective matrix is being physically disrupted by simple hydrolysis and nonenzymatic proteolysis. Thus, Vashe can physically disrupt biofilms by the aforementioned processes or by being wiped from the surface as an adjunct to debridement following application of a Vashe-soaked gauze.

In comparative studies, both in vitro and in vivo, Vashe has been shown to be as effective as other antiseptics but with less cytotoxicity than other solutions.[8,9]

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The Use of Vashe Wound Solution in Children

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Because of the non-cytotoxicity of Vashe Wound Solution and its pH equal to normal skin, it has been proven to be safe and useful in the pediatric population. Elsass[1] reported 12 cases using Vashe as an adjunct to debridement in children as young as 3 years old. Many wound cleansers are contraindicated around the eyes, ears, nose, mouth, or genitalia, which makes them not useful in small children; however, Vashe does not have those limitations. In addition, since the pH of Vashe is neither basic (as those cleansers with high percentages of sodium hypochlorite) nor acidic (as those cleansers with a pH ≤ 4), it does not sting or burn upon application to a wound.[2] This is an extremely important factor in the pediatric population.

After the report of safety in children as young as 3 years old, questions arose as to the safety of hypochlorous acid (HOCl) in younger children and infants. Premature infants (< 37 weeks) have fragile, translucent skin and are sensitive to cleansing agents (ie, hydrogen peroxide, povidone-iodine, Dakin's solution, chlorhexidine).[3] Antibacterial agents, such as silver sulfadiazine, cannot be used in infants due to the risk of kernicterus. Because of the need for high humidity in the environment, fungal infections of the skin are common. In another report, Elsass[4] used Vashe Wound Solution to treat the skin and wounds on 5 premature infants (< 37 gestation weeks) (**Figure**). Two of the 5 infants were treated with phototherapy (bili lights) to control jaundice. Wounds on the patients ranged from rashes, crusting, and eczema, to actual open wounds. The HOCl was used as baths or temporary wound soaks. No vigorous wiping was employed because of diminished cohesion between the epidermis and dermis in the preterm babies.[3]

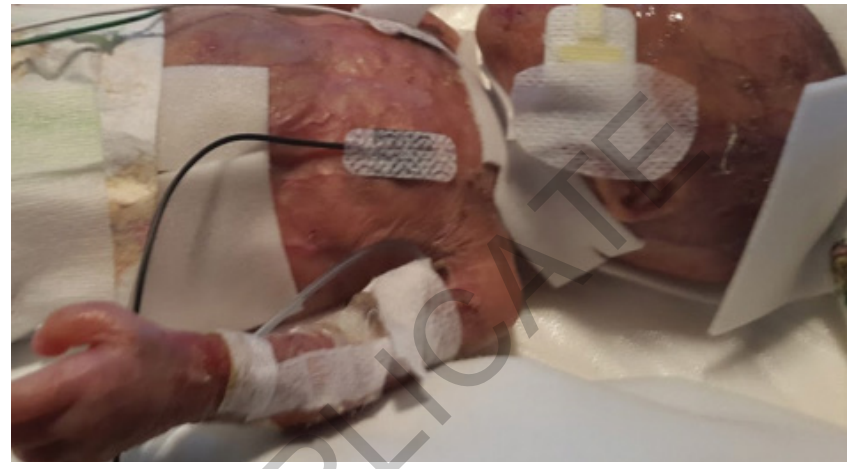

Figure. A 23-week-old gestation infant with open skin lesions and crusting at 10 days after beginning wipes with Vashe Wound Solution demonstrating complete healing.

The HOCl was well-tolerated in all 5 patients and no safety issues were reported.[4] The bacterial colonization was controlled, and fungal infections did not occur. High humidity was able to be maintained with a decrease of transepidermal water loss. Also, skin irritation from the cleansing did not occur, and bili lights could be used as indicated.

Couch et al[2] reported a cool, soothing sensation when Vashe is applied to the skin. Another advantage to Vashe is its clean, fresh, sanitary odor, which is important in a younger child whose wound may be contaminated with urine or bowel contents. The use of a hydroconductive dressing slightly moistened with Vashe around tubes, such as gastrostomy buttons, tracheostomy, gastrojejunostomy, and chest tubes, greatly decreases the occurrence of skin breakdown as a result of moisture.[2]

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Summary: The Role of Hypochlorous Acid in the Management of Burn Patients

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Thermal injuries may well be among the most complicated disease processes known. Most healthy individuals live in a symbiotic relationship with the bacteria and fungi in the environment. Human skin, particularly the epidermis, provides the first line of defense against these organisms. Once that barrier is breached, the risk of infection increases until that integrity is restored.

Burn Wounds

Management

It has long been known that early interventions aimed at decreasing infection risk in burn patients serve to increase long-term survival. Since the 1970s, the standard of care in burn wound management has included early excision and grafting of the wound.[1] The principle underlying this management is that removal of damaged, contaminated tissue followed by rapid closure of the wound decreases infectious risk. In addition, a dirty wound bed increases the likelihood that subsequent skin grafting will fail, further increasing the mortality risk for this patient population. It goes without saying that topical agents aimed at decreasing wound contamination also play an integral part in reducing the morbidity and mortality associated with burn injuries.

A 1991 study by Becker et al[2] showed that while the incidence of bacterial wound infections decreased in their center's burn patients over a 10-year period, the incidence of fungal burn wound infection remained static at 7.5%. This is possibly secondary to the fact that the main risk factors for fungal infection include increased total body surface area burn, broad-spectrum antibiotics, central lines, and decreased immune function. Additional risk is conferred to patients whose burns are extinguished by contaminated water sources, such as water found in garden hoses, as well as soil from areas known to have endemic fungus.

As it currently stands, there is a dearth of topical agents available for wound management, burn or otherwise, which are noncytotoxic.[3] Furthermore, very few antifungal and antispore-forming bacterial agents are available commercially, which is unfortunate considering these organisms' propensity to colonize burn wounds.

Evolution of Vashe Wound Solution

In wound management, regardless of etiology, a delicate interplay exists in using an antimicrobial agent strong enough to kill the many micro-organisms that may compromise a burn wound or skin graft, while at the same time minimizing the cytotoxicity that may damage the proliferating epithelial cells. Physicians have perpetually struggled to find agents that are strong enough to neutralize the microbes of a wound without being caustic to the wound bed.

Dakin's solution

After witnessing many instances of overwhelming sepsis occurring from combat wounds in World War I, an English chemist, Henry Dakin, and a French Army surgeon, Alexis Carrel, sought to find a new agent that was less cytotoxic than carbolic acid, which was widely used to clean wounds at the time. Their solution, now known as Dakin's solution, consisted of 0.5% sodium hypochlorite (NaOCl) — the main component found in household bleach. This solution was applied continuously via rubber catheters, which were inserted into the wound dressings themselves.[4] Although Dakin's solution was significantly less cytotoxic than carbolic acid, it still had many disadvantages. Unfortunately, Dakin's solution is highly basic and rendered ineffective when neutralized. Its highly unstable nature requires frequent reapplication as it is converted to water and sodium chloride (NaCl) within minutes of application. Furthermore, the chloramines generated from the NaOCl induce oxidative tissue damage and harm the wound bed itself.[5] Although many physicians have attempted to minimize the cytotoxicity of this agent via dilution, Hidalgo et al[6] reported that concentrations as small as 0.0005% are still capable of damaging cells.

Since the introduction of Dakin's solution, physicians have attempted to use other agents to minimize the bacterial burden of burn wounds, including silver nitrate, Betadine (Avrio Health LP, New York, NY), and 5% Sulfamylon (Mylan, Canonsburg, PA). Unfortunately, all of these agents are cytotoxic to varying degrees and negatively affect cell proliferation within the healing wound bed.

Hypochlorous acid

In recent years, researchers have returned to the possibility of using hypochlorous acid (HOCl) as a topical antimicrobial agent for wounds. Although HOCl was introduced as a potential wound cleaning solution during the World War I era, it failed to gain widespread use as its development was overshadowed by the introduction of antibiotics. As compared with NaOCl, HOCl is stable, has a pH that may be similar to that of human skin, and most importantly, has been shown to be noncytotoxic. Now, wound care specialists are

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returning to HOCl given its many advantages over other widely used wound care solutions.[5]

Hypochlorous acid is the final product generated within the oxidative burst pathway, thus mimicking the naturally occurring environment found within the phagosome of the neutrophil. When a neutrophil engulfs a microbe, reactions occur within the phagosome. Initially, superoxide radicals react to create a variety of end products, including hydrogen peroxide. In the presence of hydrogen and chloride, hydrogen peroxide is converted to HOCl by myeloperoxidase and superoxide dismutase.[7,8] The resulting product, HOCl, has been shown to kill the engulfed microbes through several mechanisms. Hypochlorous acid works to kill bacteria by destroying its cell wall and accomplishes its bactericidal effect via oxidation, enzymatic activity, and the inhibition of DNA synthesis.[5,8-11] Also, HOCl contains activity against fungi and their spores, as well as viruses, and perhaps most importantly, has been shown to disrupt bacterial and fungal biofilms.[12] With multiple bactericidal mechanisms working simultaneously, the potential for resistance to HOCl is decreased substantially.

Vashe Wound Solution

Vashe Wound Solution is a saline-based wound solution containing HOCl at a concentration of 0.033%. It is stable for about 24 hours, which allows for its relatively infrequent application, as compared with Dakin's solution. Instead of continuously irrigating wound dressings, gauze or Kerlix (Vitality Medical, Salt Lake City, UT) may simply be soaked in Vashe as needed. A critical difference between bleach (NaOCl) and HOCl lies in the fact that the latter is stabilized at a much lower pH, resulting in a higher proportion of the protonated ion (HOCl) being available.[5] The pH of Vashe is equivalent to that of human skin (5.1–5.5); thus, it is not irritating and does not cause pain upon application. Most importantly, research has shown stabilized forms of HOCl drastically decrease the number of bacteria in granulating wounds without resultant cytotoxicity.[10]

Extensive research has shown Vashe to be noncytotoxic, nonirritating, and nonsensitizing. Due to its pH, it can be safely used in sensitive areas, such as the mouth, ears, eyes, and genitalia. In addition, it has great utility in the pediatric setting, as its relatively neutral pH minimizes its discomfort upon application. Vashe is indicated for the cleansing and debridement of virtually any wound.

Production of Vashe

Hypochlorous acid (ie, Vashe Wound Solution) can be produced either via chemical or electrochemical means. Chemically, HOCl is created when NaOCl is acidified with dilute hydrogen chloride in the presence of NaCl. The more popular method of HOCl production consists of an electrochemical process. This method entails electrolyzing a dilute solution of NaCl until HOCl is produced. This reaction occurs in an electrochemical or galvanic cell containing both a cathode and anode, which are separated by an ion-permeable membrane. When NaCl is added to the cell, hydroxide anions are produced at the cathode and subsequently react with chlorine to produce HOCl and an oxygen byproduct.

Alternatively, Vashe may be purchased prepackaged in various sized bottles from the supplier.[5]

Vashe in burn wounds

Vashe Wound Solution is of great utility in any burn center, given its broad spectrum of antimicrobial activity and lack of cytotoxicity. It can be directly applied to a burn wound and used on grafted skin, with no risk of damage to the graft itself or granulating tissue underneath. As infection is one of the major causes of graft failure, protection of a recently grafted wound bed with Vashe has the potential to decrease the incidence of this sequela. In comparison with other agents commonly used in burn wound care, such as Sulfamylon, Vashe does not cause discomfort upon application to the wound bed. Additionally, it does not discolor the skin (like silver nitrate) and is odorless. Considering the stability of Vashe, wound care providers do not need to continuously irrigate the burn wound with it as is done with Dakin's solution. If desired, Vashe may be used as a soak and a gentle debrider in order to prepare the wound bed.[13] Moreover, Vashe can be used to treat or prevent fungal disease in the wounds as well.

Another application of Vashe Wound Solution within a burn unit is its use as a prophylactic bathing solution to decrease patient-to-patient transmission of infection. A recent study from the Arizona Burn Center at Maricopa Medical Center (Phoenix, AZ) utilized a regimen of mupirocin and daily HOCl bathing in their burn intensive care unit (ICU) in order to universally decolonize their patients. As a result, the incidence of methicillin-resistant *Staphylococcus aureus* infections in their burn ICU decreased by a statistically significant amount.[14]

Organisms

In burn injuries, the skin and its native flora are compromised significantly, leaving this patient population highly susceptible to both bacterial and fungal infections. Sequela of burn injuries, including tissue edema, which severely limits perfusion and oxygenation of the damaged tissue, and subsequent immunocompromise further predispose burn patients to both local and systemic infection.[15,16]

Microbes that commonly colonize burn wounds and cause bloodstream infections in burn patients include pathogenic Gram-positive bacteria, Gram-negative bacteria, and fungi.[17] Research has shown burn wound colonization is a fairly rapid process, with colonization of acute burn wounds with Gram-negative and Gram-positive organisms occurring as rapidly as 24 hours in one-third of burn patients.[18] Typically, fungal colonization of a burn wound follows invasion by Gram-positive and Gram-negative bacteria. Conversion from local to systemic, bloodstream infections (BSI) unfortunately are common in this population and have been reported to increase mortality rates by 4-fold.[19]

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Table. In vitro time kill assay test results

	Control	Vashe log reduction	% Kill
Methicillin-resistant *Staphylococcus aureus*	6.20	≥5.20	≥99.999%
Vancomycin-resistant enterococci	6.20	≥5.20	≥99.999%
Escherichia coli	6.28	≥5.28	≥99.999%
Acinetobacter baumannii	6.15	≥5.15	≥99.999%
Bacteroides fragilis	6.66	≥5.66	≥99.999%
Candida albicans	6.63	≥5.63	≥99.999%
Enterobacter aerogenes	6.43	≥5.43	≥99.999%
Enterococcus faecium	6.08	≥5.08	≥99.999%
Haemophilus influenzae	6.59	≥5.59	≥99.999%
Klebsiella oxytoca	6.18	≥5.18	≥99.999%
Micrococcus fetus	6.04	≥5.04	≥99.999%
Proteus mirabilis	6.40	≥5.40	≥99.999%
Pseudomonas aeruginosa	6.11	≥5.11	≥99.999%
Serratia marcescens	6.08	≥5.08	≥99.999%
S epidermidis	6.69	≥5.69	≥99.999%
S haemolyticus	6.57	≥5.57	≥99.999%
S hominis	6.68	≥5.68	≥99.999%
S saprophyticus	6.68	≥5.68	≥99.999%
S pyogenes	6.53	≥5.53	≥99.999%
K pneumoniae	6.70	≥5.70	≥99.999%
M luteus	6.04	≥5.04	≥99.999%

Results measured pathogenic colony log reductions in Vashe Wound Solution (time kill assay 15-second contact).

As the management of burn wounds continues to improve, survival rates and durations of hospital stays for burn wound survivors have increased significantly. As such, the incidence of fungal infection has risen drastically in this patient population. In 2008, Murray et al[20] reported a fungal incidence of 44% in all fatalities at their burn center, with *Aspergillus* and *Candida* being the most commonly encountered species at autopsy. Greater percentage of TBSA involved in the burn and longer length of hospital stay were both positively correlated with the incidence of fungus. In addition to nosocomial factors, burn patients are susceptible to fungal infection secondary to prolonged exposure to a single, topical antimicrobial therapy and warm wound beds that are kept perpetually moist. These factors make burn wound beds ideal incubators for fungal growth.

Antibacterial activity of Vashe

Vashe Wound Solution has been proven to possess activity against the most commonly found organisms known to colonize burn wounds. It has been shown to decrease concentrations of *S aureus*, *Streptococcus* spp, and *C albicans* by 99.999% at a kill time of 15 seconds (**Table**). Not only does it have the ability to kill a wide variety of bacteria and fungi, but it has been proven to do so rapidly and at very low concentrations. Even in dilutions of 1/64, HOCl has demonstrated the ability to exert bactericidal and fungicidal properties against several strains of *S aureus*, *P aeruginosa*, and *C albicans* in less than a minute.[21] Additionally, it has proven activity against endospore-forming bacteria, such as *Bacillus anthracis* and *Clostridium difficile*.[22]

Antibiofilm activity of Vashe

Biofilms are known to greatly complicate wound management, as they harbor several physical and metabolic properties that render them highly resistant to antibacterial agents. In the burn patient, biofilms hamper proper wound bed preparation and may prevent coverage of the damaged tissue bed or may cause later graft failure. In a recently published study by Ortega-Peña,[12] HOCl was shown to prevent and destroy both bacterial and fungal biofilms at several stages of formation. This research[12] supported the prior findings of Sakarya et al[8] in which a dose-dependent response was exhibited when HOCl was exposed to biofilms composed of *S aureus, P aeruginosa*, and *C albicans* isolates. The authors additionally demonstrated that HOCl had favorable dose-dependent effects on fibroblast and keratinocyte migration, a surrogate assay for cytotoxcicity.[8]

Antifungal activity of Vashe

As the rate of fungal infection continues to increase in hospitalized burn victims, the need for effective topical antifungals is greater than ever. In 2002, Gupta et al[21] published a comparison of the activity of several common chemical wound disinfectants and pharmaceutical antifungal sprays against commonly encountered hospital molds and yeasts. When compared with phenol, sodium dodecyl sulfate, quaternary ammonium salts, terbinafine spray, and bifonazole spray, chlorine was the only antifungal agent that rapidly inactivated all 5 clinically pertinent *Aspergillus* and *Candida* strains tested. A 1% chlorine solution killed all isolates of *C albicans, C krusei, C parapsilosis,* and 2 isolates of *A ochraceus* within 15 minutes of contact.[21]

Of great concern to the burn patient are rare, yet dangerous, nosocomial yeasts that have a predilection for the immunocompromised. *C auris* is an emerging pathogen that is almost exclusively found in the hospital setting and resistant to many antifungal agents. It is susceptible to only polyenes, triazoles, and echinocandins.[23] Perhaps the most alarming feature of *C auris* is that it has been cultured from both dry and wet surfaces for 14 days.[24,25] It goes without saying

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that this pathogen serves as a great threat to the hospitalized burn patient, especially as it may be easily spread from patient to patient. Since the first report of *C auris* in 2009, no standard cleaning regimen has been described to lessen the nosocomial transmission of this microbe. Of additional concern is finding an agent that may be used to decolonize patients in a further attempt to reduce nosocomial transmission. Chlorine-based disinfectants have been proven to have activity against *C auris* and efficacy as hand sanitizers and patient decolonization agents.[23] Although no HOCl-specific studies have been conducted to date, Vashe Wound Solution may contain activity against this organism since it has a strong activity against several *Candida* strains.[26,27] Further research is warranted in this area.

Ocular injury is not uncommon in severe chemical burns and may require the use of implantable devices, such as a Boston Keratoprosthesis (Boston KPro), in order to salvage vision. Unfortunately, Boston KPro implantation confers a high lifelong risk of fungal infection.[28] Currently, no standard of care exists for antifungal prophylaxis after Boston KPro implantation, and the antifungals used in the United States are often cost prohibitive in the developing world. A recent in vitro study by Odorcic et al[29] using 0.01% HOCl against *Acremonium kiliense, A flavus, A fumigatus, Fusarium solani,* and *Mucor indicus* showed a reduction of viable conidia by a minimum of 99% at 15 seconds. By 1 minute, the reduction increased to 99.9% or better for all species. Given these results, the authors postulated that since HOCl's relatively low cost and rapid activity against a variety of molds and yeasts commonly are known to cause ocular infections, it might be an ideal candidate for ocular infection prophylaxis in Boston KPro recipients both in the United States and developing countries.[29]

Clinical studies using Vashe

To date, very little data have been published in regard to the clinical use of Vashe Wound Solution. In 2010, Niezgoda et al[7] analyzed Vashe's role in the management of chronic wounds. In this study, 31 patients with chronic wounds received the standard of care therapy for his or her given wound, with Vashe used as an adjunct therapy. Of the 31 participants, 79% experienced complete wound healing by 90 days. A limited number of patients continued to be evaluated due to a "continuing healing process"; at the end of the study, 86% of participants experienced complete wound closure. Of the wounds that did not heal, the wound size decreased by an average of 47% at 90 days.[7]

Conclusions

In conclusion, physicians who manage wounds have long struggled to find topical solutions strong enough to decontaminate a wound bed, yet lack cytotoxic effects. Colonization of a burn wound bed poses a particular conundrum to the care provider, as infection is a major cause of graft failure, yet damage to granulating wound bed tissue is detrimental to the healing process. Vashe Wound Solution's unique activity against fungi and spore-forming bacteria further increases its utility in the burn patient, because these wounds are highly susceptible to colonization with these organisms.

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Roundtable Discussion

Dr. Robson: The previous presenters have covered many subjects regarding hypochlorous acid and specifically Vashe Wound Solution. Now we will have any further questions or comments. Several of you have mentioned the action of Vashe Wound Solution on biofilm. Dr. Hickerson, did you have any further comments on biofilm?

Dr. Hickerson: No, I think you have hit the nail on the head. We don't really kill the biofilm, but by thinking of it as a disruption, one can get in and take care of the bacteria because basically the biofilm is alive and forming a protective environment. Greg Schultz has published a lot of interesting data on biofilm and its disruption.

Dr. Robson: Dr. Moffatt, do you have anything you want to add or subtract?

Dr. Moffatt: No, your summary sounded completely accurate to me as well as what was discussed by the presenters.

Dr. Robson: I think Anna Day's articles and posters from your laboratory are very useful and we will make sure that her work is referenced in the supplement. Dr. Driscoll, do you have any comments or questions?

Dr. Driscoll: We keep coming back to a necessity of multimodal therapy for burn wounds, and the deeper the wound, the more different techniques one has to use.

Physical disruption, whether you are referring to proteins protected in a biofilm, or whether you are talking about a burn eschar, they need to be disrupted so that the activity of our antimicrobial agents can occur. That is something that we, as surgeons, cannot get around. There is no shortcut to that. We need to keep hammering that point home so that we hopefully avoid some of the inappropriate, ineffective results of a product like Vashe when used on some of these deep wounds or wounds heavily covered with a lot of debris.

Dr. Robson: Years ago, I had the idea that you ought to be able to apply something on a full-thickness burn and just selectively dissolve the eschar. We worked in the laboratory trying to do that. The problem was that anything that dissolved eschar did a lot more than just dissolving the surface nonviable tissue. To the best of my knowledge, nothing safe has been validated to just eliminate nonviable tissue while preserving the underlying viable tissue.

Dr. Robson: The question has arisen regarding the safe temperature for Vashe Wound Solution. The company has shown that the product is stable at 40°C for up to 30 days and at a temperature of 10°C for up to 120 hours. Exceeding these temperature limits changes the pH of the solution. As you decrease the pH, it is no longer Vashe Wound Solution, so you lose all of the data that have been presented to the FDA. The idea is to maintain the pH for which the patents for Vashe Wound Solution exist. The reason that the temperature questions arose had to do with environmental conditions during shipping. That is why the company did the temperature range studies.

They raised and lowered the temperatures and developed the safety ranges.

Dr. Hickerson: If a product is left on a loading dock in extreme heat, a lot of things will change.

Dr. Robson: Possibly, there should be a warning about temperature extremes. I thought that when frozen skin substitutes had to be shipped to burn centers, the problem was recognized. I thought everyone knew that if you were shipping a frozen product to a hospital or unit, someone had to be tasked with picking it up. If not, frozen products were not frozen when they got to Memphis or Phoenix.

Dr. Hickerson: Then they started shipping products on dry ice with the attendant problems that go with that. We have the requirement that such products have to arrive when Receiving is open. Receiving then has only 1 hour to notify the skin bank coordinator that the product has arrived.

Dr. Robson: So, you had to put out a specific protocol spelling out these requirements?

Dr. Hickerson: Without a doubt.

Dr. Robson: I think they devised a protocol at Maricopa County for Vashe when they first had it sitting on the loading dock too long.

Mr. Steadman (CEO of Urgo Medical): The company has revised the outside labeling on the box to remain at room temperature so that it does not sit on a loading dock too long.

Dr. Hickerson: You can only hope that the label is read and not like people in a hospital that utilize chlorhexidine gluconate ignoring label warnings not to use it around eyes, ears, nose, mouth, and mucous membranes.

Dr. Robson: Does anyone have additional comments or questions?

Dr. Driscoll: I wonder what people are using for their operating room surgical site decontamination procedures.

Dr. Robson: That's an interesting question. There are specific regulations for a skin preparation product. Vashe Wound Solution is not approved as a skin prep.

Mr. Steadman: That is correct. There is a direct guidance document available, and Vashe Wound Solution is not eligible. It is not possible to obtain that indication at this time.

Dr. Robson: There's a tremendous intraoperative use of Vashe Wound Solution. It has been reported to be very effective, but as it gains wider use, some reports are off-label. There is a group at Maricopa County who have demonstrated usefulness in treating purulent peritonitis, an idea that was published as early as 1917.

Dr. Driscoll: That particular experience has been published. Have you heard of infusing hypochlorous acid with negative pressure wound therapy devices?

Mr. Steadman: Vashe Wound Solution is currently the number two solution being used with the VeraFlo system. Following many questions, we have done material compatibility studies to show there is no material incompatibility with the VeraFlo equipment.

Dr. Hickerson: It seems like when Vashe is used with the VeraFlo system, there can be problems with occlusion. Do you know why that appears to be the case?

Mr. Steadman: We have worked with Acelity, and when they examined occluded equipment, it was their opinion that the occlusion came from proteinaceous material from the wound. They thought the wound cleansing was more effective with Vashe, and that the wound debris was clogging the tubing. There was no crystallization of the solution. The dual track pad has decreased the problem substantially.

Dr. Robson: Dr. Foster, I have a question about Dr. Matthews' work of irrigating septic peritonitis with Vashe Wound Solution. Does he use Vashe intravenously, since he quotes articles from 1917 in which hypochlorous acid was administered intravenously?

Dr. Foster: No. He has not given Vashe IV to a single patient.

Dr. Robson: I wonder since his reports are off-label, if you combined his work with the lack of cytotoxicity reported by Dr. Shupp for intraperitoneal irrigation with Vashe, an acceptable indication could be developed.

Dr. Foster: I think we can. Dr. Matthews has been using Vashe under very controlled conditions and the patients are closely monitored with very strict indications. The treatment has not been detrimental.

Dr. Robson: Mr. Steadman, is it worthwhile to see if we can bring such treatment in for approval?

Mr. Steadman: As you know, we are seeing more off-label use in these types of applications. We are also seeing off-label uses in the field of orthopedic surgery. Orthopedists use Vashe Wound Solution on some of their implants. I believe we must look for opportunities but must stay within approved indications. I do believe there are opportunities to pursue expanded indications with the FDA providing we start building the necessary clinical data.

Dr. Robson: It seems that if we build on clinical examples and provide careful safety studies, we might be able to expand approved indications. Does anyone else have any additional comments or questions? If not, this concludes the symposium.

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