The battle against bacteria is one fought daily in the delivery of wound care, and the outpatient wound clinic is certainly no exception. In fact, this setting may even be of more concern due to the nature of caring for multiple patients in the same treatment rooms in a single day. The practice of paying exquisite detail to infection control is one where we must never let our guard down as clinicians. We need to be so habitual in such methods of safety as glove use and hand hygiene that they should occur without conscious thought. Chronic wounds being what they are, the mere presence of bacteria on the surface of an open wound is expected and frequently not an impediment for the eventual closure and ultimate healing of the wound. However, it is imperative that it be eliminated as much as possible, not just for sanitation and patient-safety concerns, but since proliferation of bacteria can lead to clinical infection and the complaint of pain is frequently an accompanying presenting factor.

**FOCUS ON HYPOCHLOROUS ACID**

Wounds should be cleansed before and after debridement as well as between dressing changes. The choice of solution to be used must be driven by the goal of care and the need to clean versus the need to disinfect. A clean, granulating wound may only require isotonic saline or other non-toxic solution or cleanser to remove residue from a previous treatment or dressing. In the presence of odor, abnormal color of exudate, or other signs of increasing colonization, use of an antimicrobial product to both cleanse and reduce surface bacterial burden will be beneficial. Recently, a cleansing-and-moistening solution known as hypochlorous acid (HOCI) has been made available for clinical practice and commercial use in the forms of single-patient bottles and sprays (eg, Vashe Wound Therapy®, PuriCore, Malvern, PA; Microcyn® Skin and Wound Cleanser, Oculus Innovative Sciences, Petaluma, CA; and NeutroPhase® Wound Cleanser, NovaBay Pharmaceuticals, Inc., San Francisco, CA). A naturally occurring small molecule generated by white blood cells in the human body, HOCI can also be produced by electrolysis using a generator and specific electrolyte fluid for point-of-use care in the clinic setting (eg, Vashe). The resulting HOCI solution is stable for 72 hours in an opaque container at room temperature and for 14 days when refrigerated.

An important attribute of the human body’s immune system is its ability to instigate a rapid attack against invading pathogens by releasing highly potent oxidized molecules such as HOCI. After engulfing invading pathogens, neutrophils release an “oxidative burst” of HOCI that quickly destroys engulfed...
bacteria, viruses, or fungi. Produced by neutrophils, HOCl affects microbial cell permeability and kills microorganisms by binding to critical cell membrane components. This leads to the rupture of cell membranes and subsequent disintegration of microbial cells.

Indicated for use on diabetic ulcers, venous ulcers, stage I-IV pressure ulcers, and first- and second-degree burns, HOCl offers demonstrated efficacy in the treatment of chronic ulcers. In a pilot evaluation, Selkon et al observed ulcer pain was significantly reduced and 45% of ulcers healed in patients treated with HOCl. In vitro analysis of cell toxicity testing of keratinocytes and fibroblasts showed no negative effects. The pH of HOCl is approximately 5.4, close to the natural protective pH of human skin. Clinically, HOCl has been found to reduce or eliminate odor on contact, soften dried exudate and callus, and break down surface debris and coagulum, making both easier to remove from the wound surface without trauma.

When considering the concept of pain in wound management, the impact of wound odor and exudate can have profound effects on quality of life. Add to that the impact that a particularly malodorous wound/limb can have on the wound clinic and the potential reaction by staff and other patients, and the impact on the patient is that much more. Though not always mentioned and certainly not quantifiable, this impact on the patient’s life can unquestionably cause mental anguish or mental “pain.” Consequently, another clinical use of HOCl that one author’s clinic has found extremely effective is in its use as a soak on malodorous wounds, moist callus, legs, and fungating wounds for 10-15 minutes to dramatically reduce the odor.

LOW-FREQUENCY ULTRASOUND

Emerging technology using ultrasonic devices has shown impressive outcomes as an intervention for debridement and reducing bioburden in the wound bed. However, utilization of ultrasound is hardly a new concept to the healthcare industry. Use of ultrasound to treat medical disorders appeared in Germany in the late 1930s and in the US in the late 1940s. Therapeutic ultrasound delivers energy through mechanical vibrations in the form of sound waves at frequencies that are above the threshold of human hearing (>20 kHz). Historically, ultrasound is commonly associated with diagnostic imaging, which involves the use of high-frequency ultrasound waves with minimal physiological effects. In addition, high-frequency therapeutic ultrasound (in the range of 1-3 mHz) has been used in physical therapy, physical medicine, and rehabilitation and sports medicine for many years for the treatment of soft-tissue injuries and wounds.

In recent years, low-frequency ultrasound has been employed to impact tissues in the chronic wound bed. Different frequencies (Hz) are used therapeutically to treat and assess soft tissues. Despite ultrasound technology’s long-held presence in healthcare, low-frequency ultrasound (20-40 kHz) is a fairly recent addition to the wound care armamentarium. It includes several devices manufactured by various companies that provide different methods of delivering energy to the wound for bactericidal effects and, in some cases, immediate debridement of necrotic tissue. It is also thought to facilitate the wound healing processes. This modality provides two largely non-thermal effects — cavitation and acoustic streaming. The cavitation phenomenon may be described as the creation of miniscule gas bubbles in tissue fluid and the expansion and contraction in the size of these bubbles in tandem with the variation in the ultrasound field pressure levels. These bubbles implode at certain amplitudes of the sound waves, resulting in the formation of tiny shock waves. These locally generated shock waves, in turn, liquefy
necrotic tissue, other wound debris, and associated biofilm with no negative impact on viable soft tissue or structures.5, 10

Research has shown that such implosion-related shock waves destroy bacterial cell walls.11, 12 Using low-frequency, UAW treatment devices, the built-in lavage system may provide further reduction of cell debris and bacteria to more effectively cleanse the wound site. The acoustic streaming phenomenon may be described as “temporary disturbances in the cell membranes of the chronic wounds.” Biochemical changes, which in general are beneficial, may arise from such agitations. Included in the biochemical effects are increased cell membrane permeability, increased protein synthesis, mast cell degranulation, increased growth factor production, and enhanced nitric oxide synthetase-mediated cellular mechanisms.13-19

Ultrasound wound management can be described and delivered in various ways. UAW, also referred to as contact, thermal ultrasound, is currently available in the US as three devices (Quostic Wound Therapy System,® Arobella Medical, LLC, Minnetonka, MN; Sonic One,® Misonix Ultrasound Surgical Devises, Farmingdale, NY; and Sonoca 180, Söring Medical Technology, North Richland Hills, TX). UAW has been utilized as a wound debridement and cleaning technique for years in the United Kingdom, Russia, and Germany. This technique offers many advantages (see figures 1 and 2):

- UAW procedures can be as immediate as sharp or surgical debridement, generally requiring only topical anesthesia;
- UAW is selective for nonviable or necrotic tissue, but can be effectively used for excisional debridement;
- UAW is bactericidal at the surface and penetrates surrounding tissues;
- UAW can be performed in a variety of settings by trained personnel;
- UAW procedures allow therapy at the bedside and can be utilized at the time of surgery to provide adjunctive therapy procedures.

UAW utilizes low-frequency pulsed ultrasound directed to the wound surface and surrounding tissues via an ultrasound probe. Wound irrigation fluid is directed through an opening in the probe’s tip to administer the fluid directly to the wound surface to topically treat the wound base and to serve as a coupling medium, coolant, wound lavage, or flush. In addition to separating dead tissue from the wound bed, UAW features positive wound-healing properties including increased local tissue perfusion via vasodilation and resolution of vasospasm (thermal effect); fibrinolytic division and debridement of denatured proteins; and decreased bacterial load and stimulation of fibroblasts, macrophages, and endothelial cells.

UAW also provides thermal and non-thermal therapeutic effects related to the energy created by the ultrasound wave on the targeted tissue. The body absorbs a percentage of the ultrasound, generating heat and thermal energy as the ultrasound travels through body tissue. The degree of absorption depends on the nature of the tissue, the extent of blood flow, and the frequency of the sound wave. Thermal effects include increased blood flow, reduction in muscle spasm, increased extensibility of collagen fibrils, and a proinflammatory response.20, 21

Ultrasound treatment has been proven to eradicate surface and adjacent tissue colonization of bacteria; therefore, individuals with a history of frequent cellulitis caused by multiple resistant bacteria may benefit from the reduced need for systemic antibiotics. Collaborative work done by Pierson and Niezgoda22 has emphasized the benefits of UAW treatment: Staff at Brooke Army Medical Center, Fort Sam Houston, TX, isolated 25 highly antibiotic-resistant Acinetobacter spp. (primarily A. baumannii) from wounded Iraq soldiers. Using a previously described protocol for an in-vitro model, the bacterial suspension was set to a 0.5 McFarland standard before being serially diluted to approximately 100,000 CFU/mL. Initial colony counts were taken prior to sonocation and test solutions were treated with sonocation at 60% output in 10-second bursts, followed by 50-second cool-down periods.
until a total of 120 seconds of sonication was achieved. Aliquots were then taken and plated after each 20 seconds of sonication. Bacterial death was measured by both colony counts after 24 hours of growth and acridine orange staining using a standard protocol. After UAW treatment, a significant log decrease in bacterial load was noted, with less than 5% viable bacteria identified after 120-second treatment.

LOW-FREQUENCY ULTRASOUND

Non-contact, low-frequency ultrasound (NLFU) (MIST Therapy® System, Celleration Inc., Eden Prairie, MN), see Figure 3, utilizes acoustic/sound energy to atomize saline and deliver ultrasound energy by way of a continuous mist to the wound bed and surrounding tissue, without direct contact with the wound bed.

The sequence of events begins with electrical energy being converted to a mechanical displacement. The oscillation of the tip creates an acoustic pressure wave and atomizes the sterile saline. The saline mist supports the efficient transfer of energy and promotes coupling of energy into the wound bed. This cascade of events creates a biological response including, but not limited to, a reduction of bacteria, eradication of biofilm, down regulation of inflammatory cytokines, and cellular stimulation.

There is some common terminology used when referring to ultrasound therapy:

Frequency is defined as the number of vibrations per second. The frequency of the non-contact, non-thermal ultrasound energy is 40 kHz. That translates to 40,000 vibrations per second.

The wavelength is the distance between two equivalent points on the waveform. NLFU wavelength is 3.75 cm.

The velocity is the speed with which the wave travels through the medium. Sound energy travels 1500 m/sec in water as compared to 350 m/sec in air. The NLFU intensity measures in the therapeutic range of 0.1-0.7 W/cm².

The uniqueness of the device is the non-contact and painless delivery of ultrasound energy, with an FDA indication to promote wound healing. A recently published meta-analysis addresses the clinical effectiveness of NLFU. This analysis included eight peer-reviewed studies with heterogeneous populations that were found to be consistent in both the treatment and control groups. The data was pooled to review the effects of NLFU on wound size, volume, pain, and healing time.

Summary of findings:
• 85.2% area reduction in 7 weeks;
• 79.7% volume reduction in 12 weeks;
• 42% healed at 12 weeks (mean time to heal of 8.2 weeks);
• 79% pain reduction.

The pain reduction data found during the meta-analysis provides the basis for an interesting discussion. Pain associated with chronic wounds and wound care therapies is a continual clinical challenge in patient care. We have few interventions aside from medications to address pain. While the NLFU device is not cleared by
the FDA as a pain-reduction therapy, there have been clinical observations in which patients with painful, chronic lower-extremity wounds reported a reduction in wound pain shortly after ultrasound therapy was initiated. Samies et al. did a retrospective chart review and analysis of reported pain scores from records of 15 consecutive patients (eight women and seven men ages 28–88) with painful, nonhealing, lower extremity wounds treated for 2–4 weeks with NLFU. The mean pain scores decreased from 8.07 (± 1.91) pre-treatment to 1.67 (±1.76) post-treatment (P = 0.0003). No patients reported worsening pain after treatment commenced.

PROMOTING WOUND CLOSURE
Bacteria and chronic inflammation certainly provide the background for increased pain. Generally, bacteria/infections are clinically treated with antimicrobial scrubs and dressings and/or oral antibiotics. It has become increasingly evident that the problem within the chronic and acutely chronic wound is inflammation, bacteria, and biofilm. Serena looked at the impact of NLFU bacteria in a multicenter trial that assessed 11 patients living with Stage III pressure ulcers showing no clinical signs of acute infection.

The pre-treatment bacteria loads were >105 CFU/g tissue. There were 13 different types of bacteria cultured from pre-treatment punch biopsies. Each subject received six NLFU treatments for 2 weeks before being biopsied again. There was >93% reduction in the bacteria post-NLFU treatments. Also noted was a 26% reduction in wound size in 2 weeks.

An open-label pilot study conducted at the University of Miami of 10 refractory venous ulcers of large size to determine the effect of non-contact ultrasound on wound closure, bacterial counts and expression of inflammatory cytokines.

Researchers compared the baseline and end-of-treatment assessments and found a significant reduction in wound area, 45% (P = 0.0039) over the 4-week treatment period (see Figure 4). They also found a decline in individual and total bacterial counts; however, these differences were not significant. For all patients, there was also a trend toward reduced inflammatory cytokine expression compared to baseline levels; however, this reduction did not reach statistical significance. Interestingly, there was a correlation between healing and change in cytokine expression that showed statistical significance for TNF-α (P = 0.0395), IL-1α (P = 0.0351), IL-6 (P = 0.0508), IL-8 (P = 0.0990).

The wound team at Boston University Medical Center conducted a randomized, controlled pilot study on 12 patients living with nonhealing diabetic foot ulcers. The aim of this study was to evaluate the relationship between dose and duration of treatment by evaluating clinical changes and investigating systemic inflammatory response by quantifying cytokine activity. The patients were randomly assigned to one of three groups (NLFU 3 times per week, NLFU 1 time per week, standard of care). Group 1 showed significant wound reduction at weeks 3, 4, and 5 compared to baseline, with the greatest percent area wound reduction (PAR) 86% (P<0.05). Groups 2 and 3 showed 25% PAR and 39% PAR, respectively, but there are no statistical differences between group 2 and group 3 over time. Biochemical and histological analysis indicated a trend of reduction of proinflammatory cytokines (IL-6, IL-8, IL-1β, TNF-α, and GM-CSF), matrix metalloproteinase-9 (MMP-9), and macrophages in response to LFNC-US that is consistent with wound reduction when compared to control group subjects.

NLFU THERAPY
Currently, a research team at Northwestern University is assessing the impact of NLFU therapy on biofilm. The aim of this preliminary study is to evaluate the efficacy of NLFU in the treatment of biofilm-infected wounds using the well-established, in vivo, rabbit ear wound biofilm model.

The experiment included both a daily treatment group and one every other day. NLFU wounds from both treatments resulted in significant improvements in epithelial gap and granulation gap, as well as an increase in total granulation area. Viable bacterial counts were measured for both treatment groups relative to untreated wounds and resulted in significant improvements in wound bacterial burden relative to untreated wounds.

Controversy still exists regarding the significance of bacteria in nonhealing wounds. While healing occurs despite the presence of microorganisms, it is possible the density of microorganisms, how bacteria exist, or the presence of specific pathogens is critical to determine whether or not a wound will heal.

High microbial burden leads to the presence of neutrophils in the wound, which perpetuates an inflammatory environment with release of cytotoxic enzymes, free oxygen radicals, and matrix metalloproteinases (MMPs). Excessive MMPs degrade the extracellular matrix, may inhibit cell migration, and prevent wound closure. As a result, bacterial proliferation and colonization may retard wound healing. NLFU therapy is thought to promote healing in chronic wounds by removing bacteria and cleansing and debriding devitalized tissue, fibrin, and exudates. NLFU is also uniquely positioned to accelerate healing because of its ability to reach cells below the wound surface. This study, conducted in pigs, demonstrated NLFU’s ability to penetrate below the surface of both intact and wounded skin. Nile red dye was added to the saline solution of the NLFU and a sham device. The dye, a standard solution used in similar experiments because of its strong lipophilic properties and strong fluorescence, was mixed into the saline canister of the NLFU and sham device to achieve a final concentration of 0.01%. When fully analyzed, the concentration of dye penetration was at 3.0–3.5 mm on wounded skin and 2.0–2.5 mm on intact skin as compared to 0.35–0.50 mm on wounded skin and 0.05–0.07 mm on intact skin with a sham device.

Given the most recent developments in research projects assessing the mechanism within the wound, one should give thought to the following: The NLFU device delivers a low-pressure, high fre-
quency saline spray. One would hypothesize that the positive effects from the device are not due to shearing or mechanical forces. Instead, it may be that the ultrasonic frequency of the delivery system is disrupting the structure of the biofilm formed by the wound bacteria. This may then allow the host to overcome an otherwise difficult wound infection. Another potential, or additional, mechanism may be that NLFU is stimulating the host wound-healing response, restoring balance within the wound bed to a point that it is able to overcome the biofilm, bacteria, and/or inflammation present within the wound.

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REFERENCES


ADDITIONAL RESOURCES


