

# Ultrasound-Mediated Oxygen Delivery to Lower Extremity Wounds

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**Abstract:** Critical limb ischemia (CLI), an end result of peripheral arterial disease, remains a major clinical challenge. Wound healing in patients with CLI can be difficult due to diminished tissue oxygenation, often leading to recalcitrant ulcers and frequent limb loss. Numerous therapies, including hyperbaric oxygen therapy (HBOT), have been used to correct this regional ischemia, although often with mixed results. This case series investigates the effects of oxygen therapy delivery augmented by low-frequency ultrasound, a device that combines surface acoustic waveform (SAW) low-frequency ultrasound with hyperoxygenated saline to deliver oxygen to wounds. Participants included 7 patients (7 men, median age 63 years, all with hypertension) with CLI and full-thickness wounds. PaO<sub>2</sub> measurements were taken before (baseline), after provision of hyperoxygenated saline, and after sonification. The device was found to successfully oxygenate the wound beds; PaO<sub>2</sub> levels increased by a median of 59.7%, a maximum of 116%, and a median absolute difference peaking at 10.8 mmHg PaO<sub>2</sub> ( $P = 0.018$ ). In conclusion, the treatment increases wound oxygen levels and may be an option in CLI therapy.

**C**ritical limb ischemia (CLI), a condition that results from advanced peripheral arterial disease, retarding oxygen delivery to the affected extremity, is manifested by symptoms including constant lower extremity rest pain or nonhealing ulceration. Approximately 40% of patients with CLI require amputation of the affected limb, resulting in approximately 150,000 amputations in the United States annually.<sup>1</sup>

Oxygen is involved with several crucial steps of wound healing and is considered the rate-limiting factor of wound improvement. Serving as a nutrient for cell metabolism, phagocytes and other cells of the immune system convert oxygen into reactive oxygen species (ROS) that are then used for wound healing. Moreover, oxygen promotes angiogenesis through inducing vascular endothelial growth factor (VEGF) transcription and myofibroblast production and functions as a cofactor in collagen synthesis.<sup>2</sup>

Historically, low-frequency ultrasound (<100 kHz) has been used to increase the rate at which drugs and macromolecules are absorbed through

cell membranes and the epidermal stratum corneum. The mechanisms associated with this process are poorly understood, although they are likely due to a combination of cavitation and acoustic streaming.<sup>3-5</sup> Furthermore, therapeutic ultrasound has been shown to decrease bacterial resistance to antibiotics, suggesting enhanced permeability of micro-organisms in this setting.<sup>6,7</sup>

Normal saline solutions exhibit 6 ppm to 8 ppm of dissolved oxygen gas at sea level. By using a sparging technique, which entails the replacement of dissolved nitrogen, gas levels exceeding 24 ppm or more can be demonstrated. This gas replacement, or sparging, technique is defined by Henry's Law of Partial Pressure.

The purpose of this study was to determine whether a surface acoustic waveform (SAW) low-frequency ultrasound device paired with hyper-oxygenated saline could synergistically deliver oxygen to wounds.

#### KEYPOINTS

- Approximately 40% of patients with CLI require amputation of the affected limb, resulting in approximately 150,000 amputations in the United States annually.
- Historically, low-frequency ultrasound (<100 kHz) has been used to increase the rate at which drugs and macromolecules are absorbed through cell membranes and the epidermal stratum corneum.

## Methods

**Study population and clinical definitions.** Nine patients with leg ulcers were enrolled in this single-center feasibility study conducted over a 2-month period (August 2009 to October 2009) at Wound Healing Center (Raleigh, NC). Patients were considered for participation if the wound was full-thickness in depth and measured between 2.0 and 20.0 cm<sup>2</sup>. Inclusion criteria also included the ability to properly position an oximeter probe in the wound bed to achieve tissue oxygenation level baselines. Exclusion criteria included active wound infection or a wound with exposed muscle tendon or bone, as well as recent therapy with hyperbaric oxygen therapy (HBOT) or skin substitutes (ie, Apligraf [Organogenesis, Inc, Canton, MA]; or Dermagraft [Advanced Biohealing, La Jolla, CA]). The Duke University Institutional Review Board approved this protocol. Informed consent was obtained from all patients before study enrollment. The primary outcome of the study was oxygen concentration in the wound bed after treatment with the treatment system.

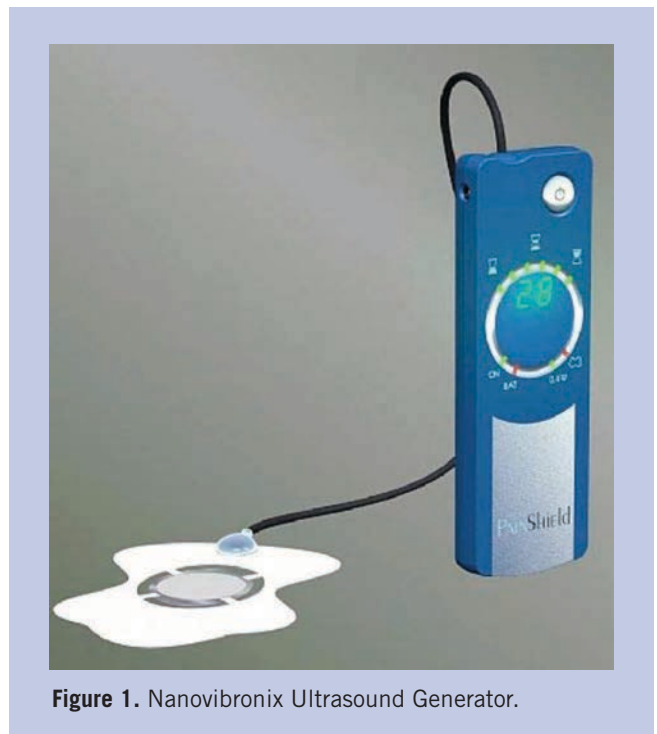


Figure 1. Nanovibronix Ultrasound Generator.

**Device.** The treatment and analysis was comprised of 3 devices: a dissolved oxygen meter/probe, a hyper-oxygenated saline instillation system, and a sonophoresis ultrasound generator. The wound tissue was sonicated at low frequencies to enhance permeability and deliver hyper-oxygenated saline, at which time PaO<sub>2</sub> levels were measured.

The devices included: 1) The low-frequency ultrasound generator (Figure 1), a diathermy device adapted for use in enhancing permeability of tissues in the body with a transducer/applicator incorporated into a patch that adheres to the skin. This device maintains the functionality of existing ultrasonic diathermy devices cited under substantial equivalency determination k081075 issued to Nanovibronix, Inc as Pain Shield™ and the substantially equivalency to the Duo-Son Ultrasonic Diathermy Device manufactured by Orthosonics Ltd, subject of k970131. This device was used for the sonication of wound tissue; 2) Oxylite (Oxford Optronix, Oxford, England), a dissolved oxygen meter manufactured used in conjunction with a fluoro-optical fiber probe with computer interface and monitoring capabilities to accurately record interstitial PaO<sub>2</sub> levels pre- and post-sonophoresis (Figure 2); 3) A SonOx instillation system (Trinity Wound Institute, LLC, Raleigh, NC) (Figure 3), used to deliver hyper-oxygenated saline to the wound bed. The system allowed for the uniform flow of fresh

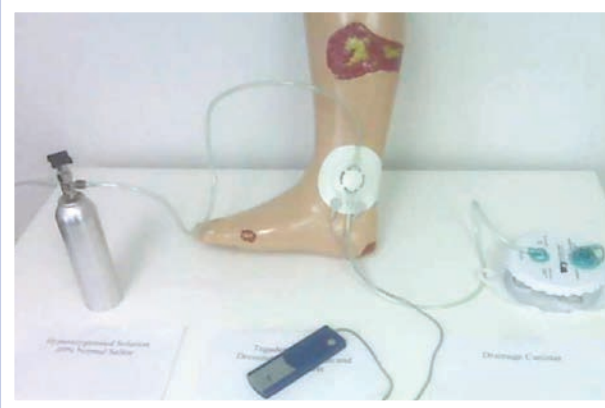


Figure 2. SonOx Ultrasound instillation system.



Figure 3. Oxylite PaO<sub>2</sub> probe.

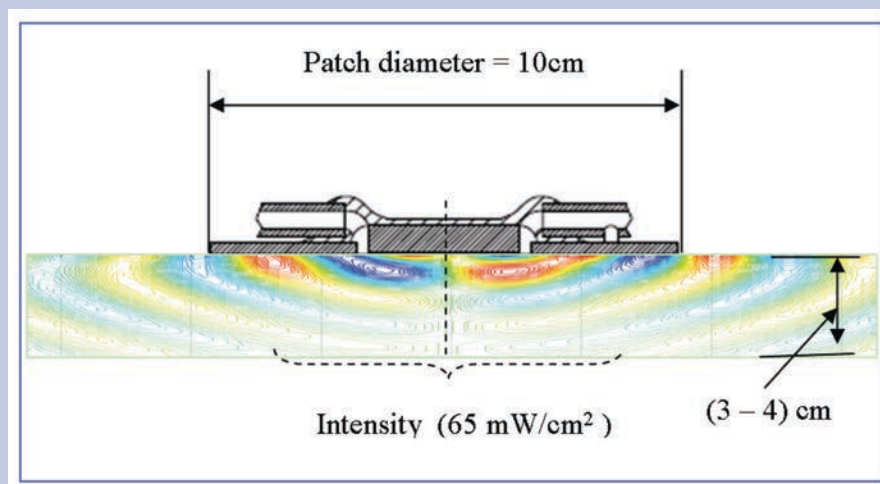


Figure 4. Profile of SAW sonication device, and pattern of ultrasound wave emission.

oxygenated sterile saline solution over the wound, while ensuring the ongoing drainage of exudate. A gas sparging technique was used to create the hyper-oxygenated saline solution, which was ultimately delivered to the wound bed.

**Procedures.** A lidocaine injection at the wound site was used for pain management. Using a location 4 mm to 5 mm from the wound edge, a 21-gauge needle was placed longitudinally beneath the wound bed at a depth of 2 mm to 3 mm. The PaO<sub>2</sub> probe then was advanced to the needle tip, and a baseline PaO<sub>2</sub> level was recorded. A control drip solution with hyper-oxygenated saline containing approximately 30 ppm of dissolved oxygen was instituted for 20 minutes without sonication and PaO<sub>2</sub> levels were recorded. The wound then was sonicated with hyper-oxygenated saline using the ultra-

sound generator for 25 minutes. PaO<sub>2</sub> levels then were recorded (Figure 4). This was followed by the removal of the probe and oxygen sensor.

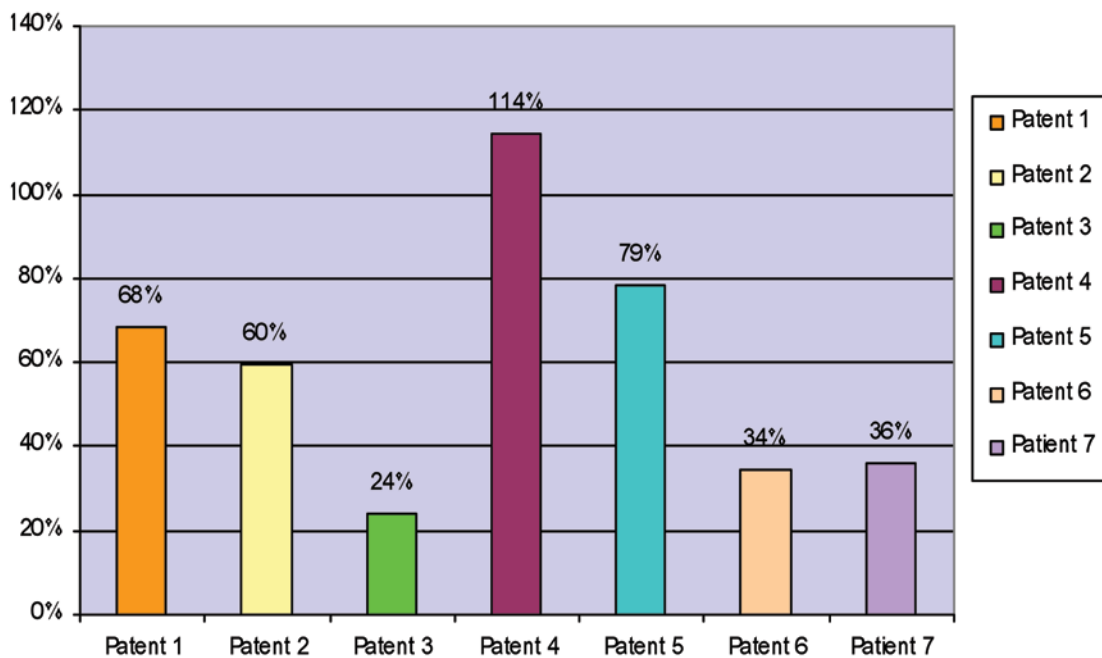
**Statistical analysis.** The Wilcoxon signed rank test was used to assess whether a statistically significant difference was noted when baseline PaO<sub>2</sub> levels were compared to post-sonication with hyper-oxygenated saline PaO<sub>2</sub> levels. Statistical significance was defined as a  $P < 0.05$ .

## Results

**Patient characteristics.** Baseline age, gender, ethnicity, and comorbidities of the 9 patients enrolled are summarized in Table 1. The majority of patients were middle-aged Caucasian men with diabetes mellitus and hypertension. No adverse events were noted. Two patients

**Table 1.** Patient demographics. Nine patients were enrolled in the study; all 9 patient demographic information are included in table. Seven patients followed through and participated in the study. Numbers represent the number of patients with the designated demographic, and percentages were calculated out of the 9 total enrolled patients.

Patient characteristics	
Characteristic	
Median age (years)	63
Men, n (%)	7 (78%)
White, n (%)	6 (67%)
Diabetes mellitus, n (%)	5 (56%)
Hypertension, n (%)	9 (100%)
Hypercholesterolemia, n (%)	4 (44%)
Current smoking, n (%)	3 (33%)



**Figure 5.** Oxygen tension ( $\text{PaO}_2$ ) increase versus baseline oxygen level. Baseline dissolved oxygen levels were considered to be hypoxic ( $>40$  mmHg) and did not increase with the control drip of hyper-oxygenated saline.  $\text{PaO}_2$  level increases ranged by 24% to 116%, with an average of 57.71% increase with active sonication. Median absolute differences peaked at 10.8 units. The Wilcoxon signed rank test, used to assess whether there was a statistically significant change from baseline to follow-up, resulted in  $P=0.18$ , indicating a significant outcome.

withdrew prior to readings. Only 7 patient's results were included.

**Primary endpoint.** Figure 5 illustrates the oxygen tension (PaO<sub>2</sub> increase versus baseline oxygen level. Baseline dissolved oxygen levels were considered to be hypoxic (>40 mmHg) and did not increase with the control drip of hyper-oxygenated saline. However, sonication increased PaO<sub>2</sub> levels by 24% to 116% compared to baseline, with a median percent change of 59.7% and median absolute difference peaking of 10.8 units mmHg PaO<sub>2</sub> ( $P = 0.018$ ).

#### KEYPOINTS

- The wound tissue was sonicated at low frequencies to enhance permeability and deliver hyper-oxygenated saline, at which time PaO<sub>2</sub> levels were measured.
- Sonication increased PaO<sub>2</sub> levels by 24% to 116% compared to baseline, with a median percent change of 59.7% and median absolute difference peaking of 10.8 units mmHg PaO<sub>2</sub> ( $P = 0.018$ ).

## Discussion

This safety and feasibility study measuring the efficacy of the treatment by the combination of using SAW ultrasound technology with hyper-oxygenated saline showed a statistically significant increase in PaO<sub>2</sub> levels in the wounds. It was the first study of its kind to evaluate whether hyper-oxygenated saline could be delivered to the wound bed via SAW ultrasound technology, resulting in elevated PaO<sub>2</sub>.

The use of oxygen therapy to reduce hypoxemia was reported in the 1960s with systemic HBOT administration<sup>2</sup>, which is currently approved by Medicare and Medicaid services for use on selected lower extremity wounds.<sup>8-10</sup>

Because oxygen is required for wound healing<sup>2,11</sup> and ultrasound technology is portable, cost-effective, and easily applicable, the combination of both may provide an effective alternative for treating CLI wounds. If proven effective, it could be a paradigm shift in the way oxygen is delivered in a clinical setting for CLI treatment. In the future, this therapy may be evaluated for the delivery of other treatment modalities, such as antibiotics and growth factors.

Many wound patients cannot tolerate, afford, or gain access to HBOT. A diseased micro-vascular system or edematous tissue promotes inadequate transportation of oxygenated blood to the wounded tissue. Ultrasound-assisted dissolved oxygen therapy provides a

potential means by which oxygen can reach the wound via the external surface of the body through the use of an active energy source rather than relying on capillaries. Oxygen is delivered directly to the wound, and the systemic side effects or logistical problems are mitigated. This approach also may aid in promoting better outcomes in patients that receive HBOT but suffer from the noted physiologic conditions that could limit full benefits of the established therapy.

Dissolved topical oxygen systems have evolved over the past 50 years. Methods that deliver topical dissolved oxygen include: catalytically produced dissolved oxygen at the wound surface; diffusible dissolved oxygen bound to a carrier such as a fluorocarbon; or reservoir of gaseous oxygen sparged through the vehicle. There may be a significant advantage to delivering oxygen topically in its dissolved form, because it is biologically available immediately upon administration. The fundamental challenge to topical oxygenation methods is to create a large oxygen gradient to allow oxygen delivery into zones of tissue hypoxia. Devices that deliver unbound dissolved oxygen with an active ultrasound energy source have demonstrated significant oxygen penetration through viable porcine tissue.<sup>12</sup>

There were several limitations to this study. The small patient sample size was the main limitation. Without knowing the clinical benefits, patients were reluctant to enroll in this study; however, even in lieu of a small sample size, the findings were robust. Additionally, the authors treated all wounds as though they were the same. The authors did not assess clinical response to improved oxygenation, such as reduction in wound size or improvement in granulation tissue.

#### KEYPOINTS

- Ultrasound-assisted dissolved oxygen therapy provides a potential means by which oxygen can reach the wound via the external surface of the body through the use of an active energy source rather than relying on capillaries.
- There may be a significant advantage to delivering oxygen topically in its dissolved form, because it is biologically available immediately upon administration.

## Conclusion

Surface acoustic waveform technology that aids in the delivery of the dissolved gas at a depth in the wound bed has proved to be physiologically effective in promoting

angiogenesis, promoting collagen formation, and fibroblast production. If this can be achieved clinically, dissolved oxygenation procedures will become complementary to systemic methods of oxygenation (hyperbaric) and will allow the treating physician greater therapeutic versatility in treating wounds, possibly in a homecare environment adjunctively or as an alternative to the HBOT for a certain populace of patients.

The ability to control duration and depth of topical oxygen delivery into human tissue will allow new strategies in individualizing patient therapy. A deeper understanding into the pharmacokinetics of dissolved oxygen administration will allow manipulation of specific healing processes. Larger studies that explore controlled oxygen therapies using ultrasound-based delivery systems based on pharmacokinetics are warranted.

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