Topical oxygen therapy results in complete wound healing in diabetic foot ulcers

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ABSTRACT

Diabetic foot ulcers (DFUs) are a significant problem in an aging population. Fifteen percent of diabetics develop a DFU over their lifetime, which can lead to potential amputation. The 5-year survival rate after amputation is 31%, which is greater than the lifetime risk of mortality from cancer. Topical oxygen is a promising technique for the adjunctive therapy of chronic wounds including DFUs, but few controlled studies exist to support its clinical adoption. The aim of this study was to compare a portable topical oxygen delivery system in patients with nonhealing DFUs to standard best practice. Twenty patients were randomized into a topical oxygen group ($n = 10$), and a nonplacebo control group with regular dressings and standard care ($n = 10$), and attended the diabetic foot clinic once weekly for 8 weeks. Ulcer surface area over time was analyzed using standardized digital imaging software. DFUs were present without healing for a mean duration of 76 weeks prior to the study. They found a significant difference in healing rate between patients receiving topical oxygen and those receiving standard care. Topical oxygen, therefore, represents a potentially exciting new technology to shorten healing time in patients with nonhealing DFUs. More prospective randomized and powered studies are needed to determine the benefits of topical oxygen, but our current results are very promising.

The worldwide prevalence of diabetes has risen from 108 million people in 1980 to 422 million people in 2014.[1] Up to 4% of diabetics develop a foot ulcer annually, and 10–15% of diabetics will have at least one foot ulcer during their lifetime.[2] These percentages translate into 16 million diabetic foot ulcers (DFUs) per year, with a significant percentage of patients requiring long-standing wound management and possible amputation of the limb. The standard of care for a DFU includes a full medical assessment, surgical intervention when indicated, and local treatment that may include debridement and wound dressings. A range of specialized topical therapies have been developed to heal these wounds, including topical growth factors, protease inhibitors, and bioengineered tissue and extracellular matrix components (reviewed in White and McIntosh[3]). Despite the application of many of these interventions, DFUs are difficult to heal and are characterized as "nonhealing" when they do not respond to standard best practice within a reasonable time frame.[4] The technological advancement in the field of topical oxygen therapy has led to the development of small, nonpressurized oxygen generating devices. These next generation topical oxygen devices are a significant improvement over earlier models and may provide a viable treatment alternative for nonhealing DFUs.

The most effective treatments are those that address the underlying cause of a condition. Oxygen is recognized as an essential component of wound healing, and is thought to promote angiogenesis and the development of extracellular matrix, increased macrophage, fibroblasts and smooth muscle cell motility within the wound,[5] and influence the inflammatory response to prevent infection (through the generation of reactive oxygen species [ROS]). We know that many DFUs occur secondary to peripheral arterial disease (PAD), which results in a state of chronic hypoxia in the tissue due to inadequate vascular perfusion. Logically, increasing oxygen levels at the wound site should result in increased healing rates through induction of the mechanisms listed above, but many patients with DFUs are not good candidates for revascularization surgery because of comorbidities, late presentation, or chronic ischemia with irreversible tissue injury. Restoring oxygen to the wound through hyperbaric or localized topical oxygen means is an alternative to revascularization surgery that addresses the underlying physiology of nonhealing chronic wounds.

Hyperbaric oxygen chambers are the most common method used to treat infection and increase oxygen availability in wounds.[6] However, hyperbaric chambers represent considerable capital investment for the institution, and the therapy itself is expensive and time-consuming for the patient. Smaller "box-type" oxygen chambers are also available, to provide localized oxygen therapy to wounds at a fraction of the cost of hyperbaric therapy. There is some evidence to support their efficacy in diabetic foot ulcers,[7,8] but they are still an onerous treatment regime for

Table 1. Inclusion and exclusion criteria for the study

Inclusion criteria	Exclusion criteria
1. Diabetic foot ulcer, which has had optimum multi-disciplinary team management for >4 weeks	1. Presence of invasive infection requiring intravenous antibiotics
2. No planned treatment for arterial disease	2. Pure neuropathic ulcer with no arterial insufficiency unless they fail to heal within 12 weeks of optimum management
3. No planned surgical intervention	3. Significant reduced immunity or high dose corticosteroids (>10 mg prednisolone) or other second line immune-suppressant
4. Patients aged >18 years.	4. Patients with a known sensitivity to any of the components of the evaluation device
5. Patients who understand the study, agree to adhere to the treatment and are able to give consent	5. Patients with known or suspected malignancy in the ulcer or surrounding tissue.
6. Patients who can be followed by the same investigating team for the whole period of their participation in the study	6. Patients who do not have the physical or mental capacity, or a significant other with the ability to change the Natrox battery pack on a daily basis
	7. Patients who present with more than 10% of the ulcer surface area covered in hard eschar
	8. Patients with ulcer surface area of more than 10x10 cm
	9. Patients who are participating in another clinical study for ulcer management
	10. Patients with a known history of poor compliance with medical treatment
	11. Patients who are unable to understand the aims of the study and not give informed consent

the patient (as they require immobility for long periods of time). These types of localized oxygen therapy may also cause regional or differential pressurization of the treated limb which may reduce perfusion.[9] The Natrox system is a small, portable oxygen concentrator which processes oxygen from air and delivers it continuously to the ulcer bed through a dressing. The advantage of this system is that it can be worn while the patient is mobile, and can be used in conjunction with many of the offloading therapies and dressings used to treat a DFU. Two small studies of the efficacy of this topical oxygen delivery system have been carried out with promising results (at the Faculty Hospital Kralovske Vinohrady, Prague, and at the Wound Healing Centre in Eastbourne, in conjunction with the University of Southampton[10]). However, given the paucity of controlled trials specifically designed to review the effects of topical oxygen on ulcer care, further studies are needed. Here we report the first randomized controlled trial to determine the effectiveness of the topical oxygen in patients with nonhealing diabetic foot/leg ulceration in conjunction with standard best practice.

METHODS

Patient recruitment

This study was approved by the Research Ethics Board at St. Michael's Hospital (Approval number 15-254c) and registered at clinicaltrials.gov (trial number NCT02599805). The Natrox Oxygen Delivery System (ODS) (InotecAMD, Burlington, ON, Canada) was granted CE Mark as a Class (II) device in June 2012. This device is also approved by Health Canada in accordance with the Medical Devices Regulations, Section 36 with a Device ID: 803987 and Device identifier: NA034 on Feb 25, 2015.

A total evaluable sample of 20 subjects were recruited into the study (of 22 subjects approached for consent) from the population routinely seen by the plastic and vascular surgeons at St. Michael's Hospital (SMH) and under the care of the diabetic foot multi-disciplinary team. Subjects were recruited in accordance with the inclusion/exclusion criteria listed in Table 1. These 20 subjects were then randomized into two groups: a control group received standard care according to best practice (iodine-based dressings, regular sharp debridement of the wound, off-loading with either total contact cast or air cast as needed [$n = 10$]), and a treatment group that received standard care plus topical oxygen ($n = 10$). The randomization of subjects was carried out by placing a piece of paper with the study ID numbers for eligible study subjects into a bag and blindly picking out subjects for each of the two groups.

The diabetic foot ulcer standards of care include:

- Full medical assessment in all cases
- Surgical operation/intervention where indicated
- Local treatment of the ulcer including sharp debridement and antimicrobial dressings.

- Off-loading standard practices (Total contact cast for hindfoot and midfoot ulcers, and removable cast walkers for forefoot ulcers).

Classification of wounds: Texas grading system

The well-established, widely used University of Texas (UT) diabetic wound classification system provides staging of varying degrees based upon descriptions and characteristics of ulcers.[11] The UT system assesses ulcer depth, the presence of wound infection, and the presence of clinical signs of lower-extremity ischemia. This system uses a matrix of grade on the horizontal axis and Grade on the vertical axis. The grades of the UT system are as follows: grade 0 (pre- or postulcerative site that has healed), grade 1 (superficial wound not involving tendon, capsule, or bone), grade 2 (wound penetrating to tendon or capsule), and grade 3 (wound penetrating bone or joint). Within each wound grade there are four stages: clean wounds (stage A), nonischemic infected wounds (stage B), ischemic noninfected wounds (stage C), and ischemic infected wounds (stage D). Ulcers were labeled infected if a purulent discharge was present with two other local signs (warmth, erythema, lymphangitis, lymphadenopathy, edema, pain). Wound depth was evaluated using a sterile blunt probe. The ability to probe to bone with the presence of local or systemic infection and suggestive radiological features provided a clinical diagnosis of osteomyelitis. The diagnosis of lower-extremity vascular insufficiency was made clinically on the basis of absence of both pedal pulses of the involved foot and/or an ankle-brachial pressure index of <0.9.

After wound debridement each ulcer was graded and staged according to the above criteria. Both the Natrox™ treatment group and the control group had ulcers from Grade Ia to Grade IIId at baseline.

Demographics and outcome measures

The following demographic information was collected at baseline: gender, age, smoker/nonsmoker, BMI, number of active ulcers, and other medical conditions. Baseline photographs of the ulcer were taken 30 cm away from the wound. After sharp wound debridement with a sterile scalpel, the wound's surface area was calculated by measuring the maximum perpendicular length and width. For each patient, the ulcer was graded and staged by a clinician. For all subjects randomized to the control group, standard prescribed wound dressings were applied as described. For all subjects randomized to the Natrox treatment group, the Oxygen Delivery System (ODS) was placed directly on the wound surface, and attached to the active Natrox Oxygen Generator using the tubing provided.

Study time points

Subjects continued to attend the diabetic foot clinic at SMH on a weekly basis. At the time of this visit all subjects received wound irrigation, sharp debridement, an appropriate wound dressing and any necessary off-loading as per best practice care. Photographs and wound measurements were taken from all subjects at this visit. The topical oxygen treatment group differed because they had a topical oxygen device applied (Figure 1A). All subjects in the treatment group had their ulcer irrigated if appropriate and a new, sterile oxygen delivery pad applied each week. Prior to the reapplication of the topical oxygen device, a photograph at 30 cm away from the wound was taken. This procedure at each clinic visit was repeated for 8 weeks or until the wound was healed. After the 8-week period of treatment in the study, the topical oxygen delivery device was removed and the subjects continued with standard care. Subjects in the control group continued to receive best practice care along with photographs and wound measurements taken after debridement at each weekly visit. For all subjects at each clinic visit, a case report form was completed detailing the prescribed dressing regime, method of wound bed preparation/debridement of the wound, presence of infection, local swelling, erythema, local tenderness or pain, local warmth or purulent discharge.

Statistical analysis

Data was examined for normality with a Kolmogorov–Smirnov test. For normally distributed data, repeated measures were assessed with independent *t*-tests, one way ANOVA with repeated measures, or ANOVA with Greenhouse–Geisser correction. Statistical analysis was performed with SPSS Statistics (IBM, v22, 2013, Armonk, NY) with $p < 0.05$ was deemed significant.

RESULTS

In this study evaluating topical oxygen therapy for the treatment of hard-to-heal wounds, all subjects received best practice care as previously described. No subjects were lost to follow-up. One subject in the topical oxygen treatment group decided to discontinue treatment part way through the study following an adverse bleeding event unrelated to the treatment. One subject in the control group had a wound that was significantly larger than all other wounds in the study (7.5 cm^2 vs. a mean of 1.37 $cm^2 \pm 0.95$ in the treatment group), and was discarded as an outlier in our analysis. This resulted in nine subjects in the topical oxygen treatment group and nine subjects in the Control group for subsequent data analysis.

Patient demographics

The control and treatment groups were age-matched (mean age of topical oxygen treatment group = 57 ± 9.5 years vs. 58 ± 9.5 years in the control group [$p > 0.5$, ns]). The study population was predominantly male (85%). Of the subjects in the topical oxygen group, 30% of subjects were smokers, vs. 20% of patients in the Control group. In the topical oxygen treatment group, one subject had coronary artery disease, one subject had dyslipidemia and two patients had hypertension. In the Control group 2 patients had hypertension. At baseline measurements, the control and treatment groups had similar Hba1c and Ankle-Brachial Index (ABI) values. Mean Hba1c of $8.6 \pm 2.3\%$ in the topical oxygen treatment group vs. $7.3 \pm 0.5\%$ $p > 0.5$, ns. Mean ABI values of 1.10 ± 0.19 in the topical oxygen group vs. 0.96 ± 0.20, $p > 0.5$, ns).

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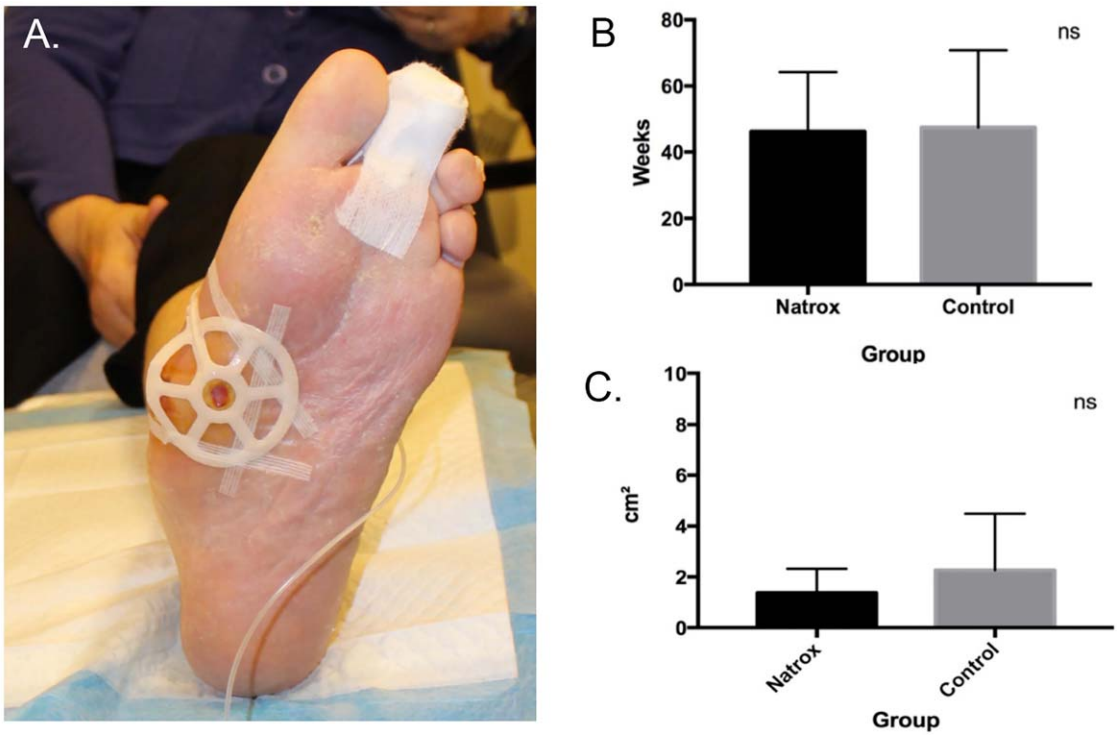

Figure 1. Study at baseline. (A) Subject in the treatment group with a Natrox oxygen delivery device in place. (B) Mean ulcer duration at baseline. The treatment and control groups were well-matched, as there was no significant difference in mean ulcer duration between groups. (C) Wound surface area at baseline. The control and treatment groups were also well matched with respect to wound surface area, as no significant difference was found between wound surface area at baseline. [Color figure can be viewed at wileyonlinelibrary.com]

Wound healing

At baseline, there was a nonsignificant difference in mean wound surface area between both groups (topical oxygen treatment group mean area at baseline of 1.37 $cm^2 \pm 0.95$ vs. the mean area in the control group of 1.68 ± 1.31, $p > 0.5$) (Figure 1B). The mean duration of the wound prior to enrollment in the study was also well matched between groups (47.4 weeks $\pm$ 23.4 in the topical oxygen treatment group and 46.2 weeks $\pm$ 17.9 in the control group [ns, $p < 0.18$]) (Figure 1C).

Assessment of wound closure rate

Using topical oxygen therapy, we saw a significant difference in the healing rate of those subjects receiving topical oxygen. Grade I ulcers in both Control and topical oxygen treatment groups all healed completely in the 8 week treatment period (Figure 2A; complete wound healing defined as a wound surface area of 0 cm^2) using our best practice standard of care. However, a noticeable difference in healing rate was seen between control and treatment groups with more advanced ulcers. While none of the Grade II wounds healed in the 8-week treatment period in the control group, 100% of ulcers treated with topical oxygen therapy completely healed in the same period (Figure 2B). Topical oxygen was also effective in healing Grade III wounds: 50% of grade III ulcers completely healed in the 8-week treatment period on topical oxygen therapy, while none of the comparable ulcers healed in the same time period in the control group (Figure 2C). Grade II and Grade III ulcers that healed had been open for approximately 46 weeks, and ulcers that did not heal were very severe, involving bone (Grade III).

Assessment of wound size

Using a repeated measures ANOVA with Greenhouse–Geisser correction, we found that topical oxygen treatment significantly decreased mean wound area size from baseline values ($F(2.238,20.146) = 8.885$, $p < 0.001$). In the topical oxygen treatment group, there was a significant difference in wound area size between baseline measurements and measurements taken at Week 2 ($p < 0.016$), Week 3 ($p < 0.09$), Week 4 ($p < 0.002$), Week 5 ($p < 0.001$) Week 6 ($p < 0.001$), Week 7 ($p < 0.001$), and Week 8 ($p < 0.001$). There was no significant difference found between baseline and Week 1 wound measurements in the topical oxygen treatment group (Figure 3B). Using the same statistical analysis, the mean scores for the control group for wound area size were not statistically significantly different ($F(1.186,10.674) = 1.447$, $p < 0.262$). While some wounds in the control group were indeed healed with our best practice standard of care, topical oxygen therapy was appreciably different in its effectiveness as an adjunctive therapy on a case-by-case basis (Figure 4).

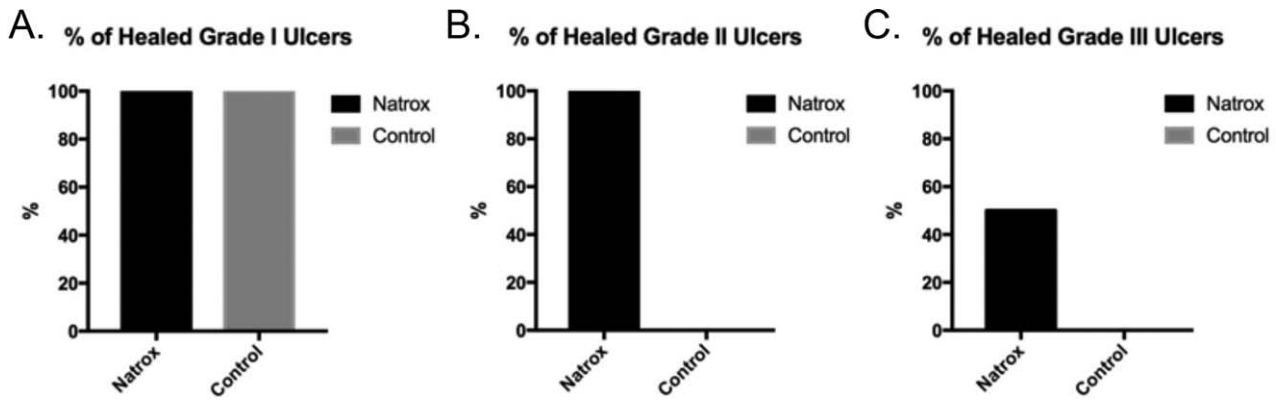

Figure 2. Percentage of healed chronic DFUs in our study. Wounds were classified according to the University of Texas System before treatment. A wound was deemed "healed" when its measurement was 0 cm^2.

Assessment of wound characteristics: Qualitative observations

Wounds treated with topical oxygen therapy were qualitatively different from the control group. The plastic surgeon treating these wounds noted clinically that the amount of wound exudate increased significantly in in the first two weeks of treatment with topical oxygen, preceding the significantly increased rate of closure of these wounds. During this therapy period, foam dressings were required to address the increase in exudate. This increase in exudate did not preclude the patient from total contact casting. Also, in total contact casting the small tubing attached to the ODS had to be protected in a foam dressing to ensure there was no risk of pressure necrosis (Figure 5).

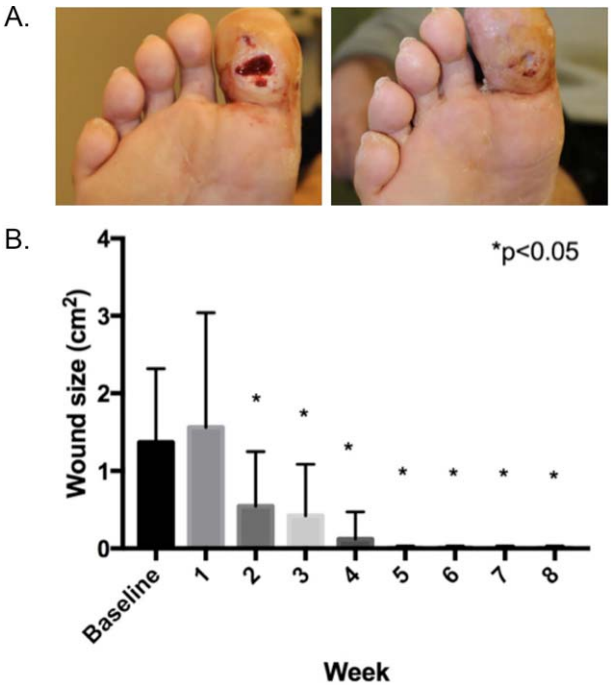

Figure 3. Wound progression over time in the Natrox treatment group, shown qualitatively (A; images shown are of the same ulcer at week 0 (left) and week 8 (right)) and by mean surface area (B). [Color figure can be viewed at wileyonlinelibrary.com]

DISCUSSION

Oxygen is essential for wound healing, and plays a role in energy metabolism, angiogenesis, the generation of ROS, infection control, signaling and construction and remodeling of the extracellular matrix (reviewed in Dissemond et al.[12]). We found a significant difference in wound healing rates in the topical oxygen treatment group compared with standard best practice care, including 80% of Stage II ulcers and 50% of stage III ulcers healed with topical oxygen, while no comparable ulcers healed when treated with standard care alone.

Previous work has shown that cells in the wound microenvironment upregulate the expression of enzymes to convert oxygen to ROS, which may act as a signal to modulate a wide variety of cellular responses.[5] Further, angiogenesis has been shown to be a critical aspect of the wound healing response, and is primarily stimulated by vascular endothelial growth factor (VEGF) which requires ROS for its action.[13] Work both in vivo and in animal models have shown that VEGF is increased in endothelial cells and the angiogenic chemokine interleukin-8 (IL-8) is secreted by macrophages in hyperoxic conditions.[14–16] Oxygen treatment has been shown to increase VEGF protein expression in wounds in vivo[17] and may trigger the differentiation of fibroblasts to myofibroblasts, cells responsible for wound contraction and healing. Finally, collagen deposition is known to be a fundamental step in wound healing that provides the matrix for angiogenesis and tissue remodeling. There are several steps in collagen synthesis that are oxygen dependent, and it has been shown in in vivo models and in human subjects that supplemental oxygen may enhance collagen synthesis and thus tensile strength.[18–20] Despite the essential role of oxygen in wound healing, the best method of supplemental oxygen delivery remains controversial. While the use of hyperbaric chambers is more common in Europe, the cost and lifestyle barriers associated with their use, coupled with the potential for significant side effects (middle-ear

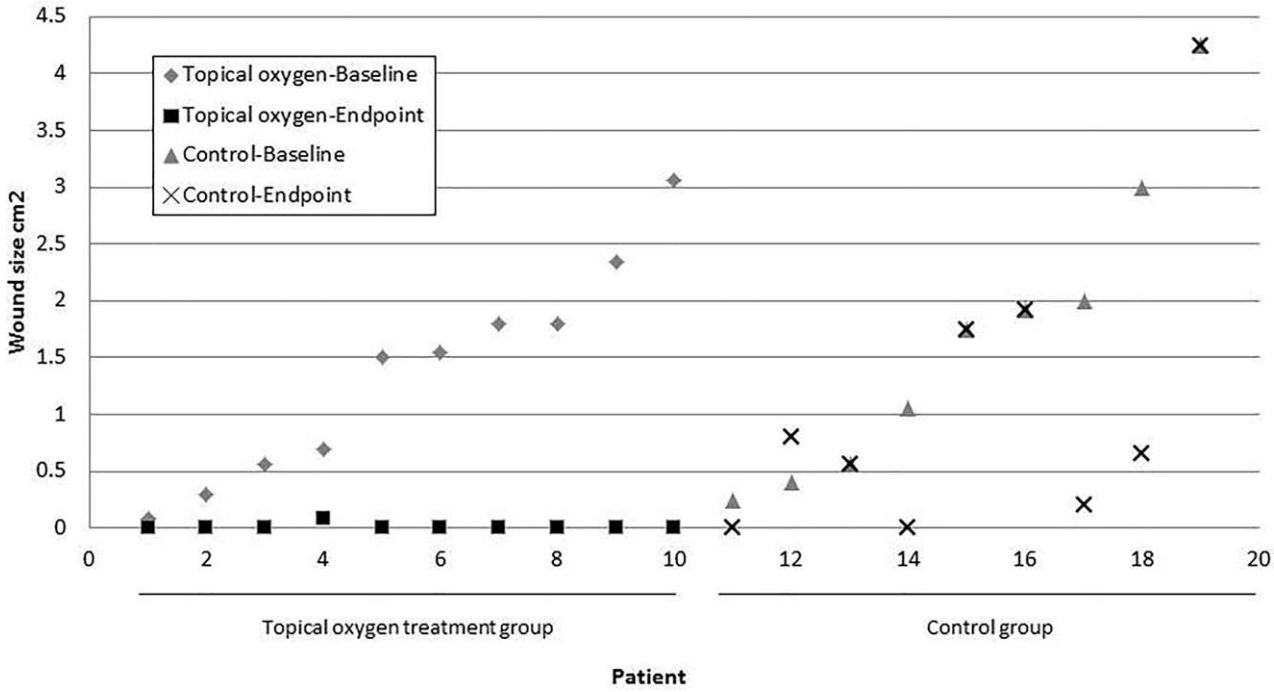

Figure 4. Wound surface area for each patient included in the study at baseline and at the end of the 8-week treatment period. Patients in the topical oxygen treatment group did universally well, and even large wounds were closed by the conclusion of the study period. While some wounds in the control group responded well to our best practice standard of care, many did not respond to treatment (and one grew over the course of the study). Topical oxygen appears to be an excellent adjuctive therapy to help treat wounds that do not respond to standard best practice care.

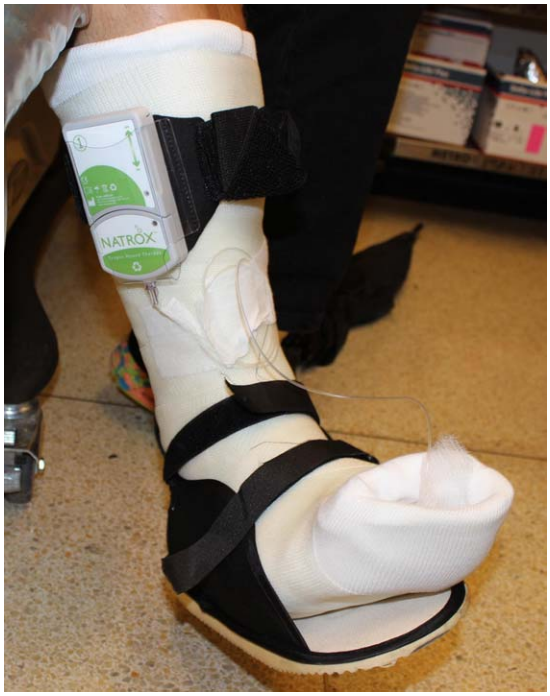

Figure 5. A Natrox device fitted under a Darko boot for offloading purposes.

damage, tympanic membrane rupture, lung failure, lung edema, and seizures) have prevented their universal acceptance. Topical oxygen offers the advantage of being cheaper, more flexible, and avoiding the potential for side effects of systemic hyperbaric oxygen, and our results support its use in the adjunctive treatment of DFUs.

LIMITATIONS

This study is a randomized control trial to investigate the utility of the specific topical oxygen device. More work needs to be done for investigating topical oxygen in general as an adjunctive therapy for DFUs. The RCT is underpowered and single centered but has created the foundation to power a multicentered non–placebo control trial. The wounds evaluated in this study were small, and a follow-up study examining the effects of topical oxygen on a larger number of patients with larger wound sizes could build upon the positive results we experienced with topical oxygen in this pilot study.

In conclusion, overall, 30% of subjects in the Control group completely healed within the 8-week study period. The subjects who healed all had Grade I ulcers, which were most likely to heal with standard care. In contrast, 90% of subjects in the topical oxygen therapy group completely healed within the 8-week study period. Topical oxygen had a significant effect on the wound size and healing rate of even the most severe Grade III ulcers seen in our study. The

results of our pilot RCT suggest that delivering a continuous flow of oxygen to a chronic wound via the topical oxygen can have a powerful effect on healing.

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