two2
MULTI-MODALITY OXYGEN

TOPICAL WOUND OXYGEN THERAPY IN THE TREATMENT OF CHRONIC AND COMPLEX WOUNDS

A Compendium of Evidence 2020

Advanced Oxygen Therapy Inc.

Advanced Oxygen Therapy Inc.

two2 MULTI-MODALITY OXYGEN

TWO$_2$ Selected Evidence

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THE JOURNAL OF CLINICAL AND APPLIED RESEARCH AND EDUCATION

VOLUME 43 | NUMBER 3

Diabetes Care®

MARCH 2020

American Diabetes Association®

ISSN 0149-5992

MARCH 2020

Diabetes Care®

In This Issue of *Diabetes Care*

By Max Bingham, PhD

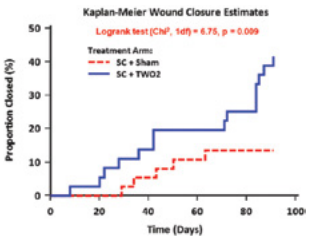
Global Mechanisms Proposed for Cardioprotective Effects of SGLT2 Inhibitors

The beneficial effects of sodium–glucose cotransporter 2 inhibitors (SGLT2i) in type 2 diabetes are mostly attributed to their ability to enhance glucose excretion and lower hyperglycemia. But they can also promote positive cardiovascular outcomes. Less clear is quite how they manage to achieve the effects, and although many hypothetical mechanisms exist, they only partly explain what might be going on. Avogaro et al. (**p. 501**) attempt to bring the different strands of evidence together and propose a hypothesis that suggests SGLT2i might modify the trajectory of cell responses to high glucose levels from one of defense to dormancy. They suggest this might be the mechanism that explains the cardiac and renal protective effects of SGLT2i treatments. On that basis they call for dedicated studies to test the hypothesis to ultimately gather the support needed for human studies. They explain that high blood glucose is effectively a toxic environment that likely shifts cell responses to a state of defense characterized by immune responses, anabolic metabolism, inflammation, adiposity, and also cardiovascular events. In contrast, they suggest that switching to a dormancy program would curtail many of these issues and that evidence suggests that SGLT2i may actually be able to force this switch—effectively explaining the positive cardiorenal outcomes of the trials. They acknowledge that most of the cited evidence comes from animal studies but suggest that, together with the more limited human data, the evidence points towards SGLT2i having a dormancy effect at a cellular level. Commenting further, author Angelo Avogaro told us: "There is still a lot to be understood about what SGLT2i do to humans beyond their glycosuric effects. Many hypotheses have been proposed, but we found it fascinating that they may switch the milieu of the cells to a state similar to that observed in mammalian animals during hibernation. If this is the case, this evolutionary hypothesis should be rigorously tested in future studies."

Avogaro et al. Reinterpreting cardiorenal protection of renal sodium–glucose cotransporter 2 inhibitors via cellular life history reprogramming. Diabetes Care 2020;43:501–507

Oxygen Therapy Improves Diabetic Ulcer Wound Healing: RCT Data

Treating diabetic foot ulcers for 12 weeks with a topical wound oxygen therapy in addition to standard care increases the likelihood that they heal, according to Frykberg et al. (**p. 616**). Specifically, they found that the therapy resulted in a >4.5-fold increased likelihood of healing compared with placebo and notably could be administered at home by patients. The results come from a double-blind randomized controlled trial (RCT) that compared an oxygen treatment approach (Topical Wound Oxygen [TWO2]) or placebo (circulating air) delivered via a device called a HyperBox (AOTI Ltd., Galway, Ireland). Both approaches were applied on top of standard care for wounds, which were long-standing and had not healed prior to the trial. The company-sponsored trial was stopped early (as planned) after the active treatment showed clear success in healing wounds compared with placebo. Seventy-three individuals had been enrolled up to that point. The primary outcome was the percentage of ulcers achieving 100% healing at 12 weeks. The authors found that the active treatment had a closure rate of nearly 42%, while the placebo had a closure rate of 13.5%. This resulted in an odds ratio of ~4.5, which was statistically significant, and it increased to 6.0 once ulcer grade was accounted for. Additionally, more than half of ulcers were closed at 12 months after the active treatment but only about one-quarter following placebo. Quality of life measures also improved more following the active treatment. There were high compliance rates in both groups, and no device-related adverse events were experienced in either group. Commenting further, author Robert Frykberg told us: "We believe that in this rather robust double-blinded RCT we have clearly demonstrated the positive effects of cyclical, pressurized topical oxygen therapy in the healing of chronic diabetic foot ulcers. Accordingly, we now have the evidence required to recommend the use of this therapy as an adjunct to good standard care for the management of difficult-to-heal diabetic foot ulcers."



Kaplan-Meier curve showing the separation between placebo (SC + Sham) and active therapy (SC + TWO2) study groups throughout the 12-week trial. SC, standard care.

Frykberg et al. A multinational, multicenter, randomized, double-blinded, placebo-controlled trial to evaluate the efficacy of cyclical Topical Wound Oxygen (TWO2) therapy in the treatment of chronic diabetic foot ulcers: the TWO2 study. Diabetes Care 2020;43:616–624

A Multinational, Multicenter, Randomized, Double-Blinded, Placebo-Controlled Trial to Evaluate the Efficacy of Cyclical Topical Wound Oxygen (TWO2) Therapy in the Treatment of Chronic Diabetic Foot Ulcers: The TWO2 Study

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Diabetes Care 2020;43:616–624 | https://doi.org/10.2337/dc19-0476

OBJECTIVE

Topical oxygen has been used for the treatment of chronic wounds for more than 50 years. Its effectiveness remains disputed due to the limited number of robust high-quality investigations. The aim of this study was to assess the efficacy of multimodality cyclical pressure Topical Wound Oxygen (TWO2) home care therapy in healing refractory diabetic foot ulcers (DFUs) that had failed to heal with standard of care (SOC) alone.

RESEARCH DESIGN AND METHODS

Patients with diabetes and chronic DFUs were randomized (double-blind) to either active TWO2 therapy or sham control therapy—both in addition to optimal SOC. The primary outcome was the percentage of ulcers in each group achieving 100% healing at 12 weeks. A group sequential design was used for the study with three predetermined analyses and hard stopping rules once 73, 146, and ultimately 220 patients completed the 12-week treatment phase.

RESULTS

At the first analysis point, the active TWO2 arm was found to be superior to the sham arm, with a closure rate of 41.7% compared with 13.5%. This difference in outcome produced an odds ratio (OR) of 4.57 (97.8% CI 1.19, 17.57), $P = 0.010$. After adjustment for University of Texas Classification (UTC) ulcer grade, the OR increased to 6.00 (97.8% CI 1.44, 24.93), $P = 0.004$. Cox proportional hazards modeling, also after adjustment for UTC grade, demonstrated >4.5 times the likelihood to heal DFUs over 12 weeks compared with the sham arm with a hazard ratio of 4.66 (97.8% CI 1.36, 15.98), $P = 0.004$. At 12 months postenrollment, 56% of active arm ulcers were closed compared with 27% of the sham arm ulcers ($P = 0.013$).

CONCLUSIONS

This sham-controlled, double-blind randomized controlled trial demonstrates that, at both 12 weeks and 12 months, adjunctive cyclical pressurized TWO2 therapy was superior in healing chronic DFUs compared with optimal SOC alone.

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Received 8 March 2019 and accepted 3 September 2019

Clinical trial reg. no. NCT02326337, clinicaltrials.gov.

This article contains Supplementary Data online at https://care.diabetesjournals.org/lookup/suppl/doi:10.2337/dc19-0476/-/DC1.

**A complete list of the TWO2 Study Group collaborators, Steering Committee, and Data Monitoring Committee can be found in the Supplementary Data online.*

This article is featured in a podcast available at https://www.diabetesjournals.org/content/diabetes-core-update-podcasts.

See accompanying article, p. 515.

With the growing worldwide prevalence of diabetes there has been a resultant increase in the incidence of diabetic foot ulcerations (DFUs) with attendant morbidity, mortality, and health care costs (1–3). Common diabetes comorbidities including peripheral neuropathy, deformity, and peripheral arterial disease (PAD) are among a number of well-established risk factors for DFUs (2,4). These person-level conditions when combined with numerous underlying cellular or metabolic and ulcer-related factors (hypoxia, inflammation, bioburden, etc.) will quite frequently lead to impaired wound healing and to possible amputation (5,6).

Over the last decade it has become clear that basic standards of care for DFUs mandate rigorous attention to proper debridement and off-loading (7–9). While a number of new adjunctive therapies have become available, including growth factors, cellular and acellular tissues, topical negative pressure, oxygen therapies, etc., most therapies suffer from inadequately designed or nongeneralizable studies that cannot attest to their efficacy, safety, and cost-benefit (1,10,11).

Oxygen is an essential component in the wound-healing cascade. Energy metabolism (ATP synthesis), reactive oxygen species generation, redox signaling, H_2O_2 production, antioxidant generation, collagen synthesis, deposition of extracellular matrix, VEGF gene expression, and angiogenesis are among processes dependent on a sufficient supply of oxygen for their activities (12–15).

Hyperbaric oxygen therapy (HBOT) has been studied extensively for its efficacy in healing DFUs and amputation prevention, but despite several recent randomized clinical trials, the results remain inconsistent regarding its effectiveness in healing DFUs (10,16–19). Topical oxygen therapies (TOTs), used in clinical practice for >50 years, supply oxygen directly to the hypoxic wound surface without the potential complications posed by HBOT (13,15,20,21). Despite long-standing clinical evidence supporting the effectiveness of topically applied oxygen for chronic wounds, hyperbaric oxygen proponents have raised concerns about such benefits without systemic hyperoxygenation (22).

To study the effect of topically administered oxygen on cutaneous wounds, Fries et al. (23) conducted a controlled porcine dermal wound-healing experiment. They found that topical oxygen increased the wound tissue partial pressure of oxygen (PO_2) levels 10-fold after 4 min and that repeated treatments accelerated wound closure compared with control (air-exposed) wounds. Histological examination showed a stronger presence of VEGF, signs of improved angiogenesis, and more advanced remodeling with better quality collagen. Their findings suggest several biological mechanisms for the enhanced healing found in other topical oxygen studies. While numerous reports have similarly suggested the potential benefits of topical oxygen in healing chronic wounds, its effectiveness in healing DFUs remains disputed due to a combination of poorly designed studies, inconsistent results, and the paucity of robust investigations through randomized controlled clinical trials (RCTs) (15,24–26).

In recognition of the need for more rigorous studies of this therapy, a randomized, double-blinded, sham-controlled clinical trial was designed to explore the efficacy of cyclical pressurized Topical Wound Oxygen (TWO2) therapy in healing refractory DFUs that had failed to heal with optimal standard of care (SOC) alone. We herein present the results of the TWO2 diabetic foot ulcer study.

RESEARCH DESIGN AND METHODS

Study Design

The TWO2 study was designed as a prospective, multinational, multicenter, double-blinded, placebo-controlled, randomized clinical trial with 17 diabetic foot centers participating across the U.S., U.K., France, Germany, and Luxembourg. The protocol was approved by the governing institutional review or local ethics board of each of the participating centers throughout the U.S. and Europe. The study was performed in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines of the International Conference on Harmonization. Written informed consent was provided by all participants prior to performance of study procedures. An independent data monitoring committee and a study steering committee were established to monitor the conduct and analysis of the study.

Sample Size and Design Rationale

Limited information was available on RCTs looking at the efficacy of cyclical pressurized topical oxygen for healing DFUs. Aburto and Frye (27), in a randomized study of topical oxygen, demonstrated better healing in DFU patients after 90 days (90% vs. 40%) compared with the control group. Blackman et al. (20) enrolled 28 patients with DFUs and obtained a similar result (82.4% vs. 45.5%). In combining the results of these two studies, the control group achieved a healing rate of 9 of 21 (42.8%), and in the active group healing occurred in 23 of 27 (85.2%). Using these figures, we would anticipate a tentative expected control rate of 43%, and it was proposed that a conservative estimate of difference between groups would be half that experienced in these trials at 21%. In order to address the unknown outcomes, we used a group sequential design with three predetermined analysis points. With three analyses, the level of significance needed to be adjusted to maintain the integrity of the analysis. The Pocock stopping boundary method requires a more stringent P value threshold ($P < 0.022$) at each of the three analysis points to achieve an overall probability of $P < 0.05$ at the final evaluation. For achievement of a minimal level of significance between study arms, it was calculated that 110 patients would be required in each study arm ($n = 220$). The resultant analyses would therefore be performed after one-third (73), two-thirds (146), and finally all (220) enrolled patients completed the active phase of the study. Since analysis would be exclusively of the intention-to-treat (ITT) cohort, all patients would be analyzed as per the 12-week primary end point (healed vs. unhealed). Furthermore, no up-rating of this sample size was made to take into consideration patients lost to follow-up.

Patients

Inclusion criteria for participation in the trial were as follows: patients with type 1 or 2 diabetes with nonhealing, full-thickness, University of Texas Classification (UTC) grade 1 or 2 DFU measuring ≥ 1 cm^2 and <20 cm^2 post-debridement. All ulcers included were to be between 4 weeks and 1 year in duration and to have been receiving standard care for at least 4 weeks. Patients with modest limb ischemia were permitted with an ankle brachial index (ABI) >0.7. To account for falsely elevated ABI measurements (7), we performed

a secondary confirmatory measurement of distal perfusion adjacent to or distal to the index ulcer in all patients, including a transcutaneous oxygen pressure ($TcPO_2$) >30 mmHg, skin perfusion pressure >30 mmHg, toe pressure >30 mmHg, or a Duplex ultrasound showing biphasic waveforms below the knee. Detailed study enrollment criteria can be found in Table 1.

Randomization

Patients were randomly assigned in a 1:1 ratio double blinded to either the SOC plus sham therapy (SC+Sham) arm or to an SOC plus active TWO2 therapy (SC+TWO2) arm. The randomization list of 220 codes in A or B format was generated by the blinded statistician using a random permuted block design, with blocks of 2, 4, 6, and 8. Study arm allocation was randomly assigned by a centralized study coordinator for each patient at the randomization visit.

Interventions

All patients were recruited as outpatients in participating wound care centers. At the screening visit and after obtaining informed consent, the patient's wound was sharply debrided and digitally photographed. All patients were then provided with the same study foam dressings and hydrogel (Kendall; Covidien), instructions, and the study off-loading device (Optima Diab; Salvatelli srl, Civitanova Marche, Italy). After a run-in period of 2 weeks, patients returned for their randomization visit. Only if the wound area reduction was <30% were patients subsequently randomized double-blind into either the active (SC+TWO2) or sham (SC+Sham) study arm.

The U.S. Food and Drug Administration–cleared, CE-marked TWO2 therapy device (HyperBox; AOTI Ltd., Galway, Ireland) operates by inflation of a single-use extremity chamber over the patient's limb; then, humidified oxygen is cycled between 10 mb and 50 mb within the chamber. A 10 liters per minute oxygen concentrator was used to provide the oxygen supply rather than oxygen cylinders.

Both the active and sham devices looked and operated identically. However, the sham device did not deliver pressurized oxygen into the extremity chamber, even though values displayed on the device controls looked as if this was being performed. The sham treatment therefore consisted only of unrestricted nonpressurized ambient room air in the nonocclusive extremity chamber.

Delivery, installation, and training on the use of the blinded study device was performed by blinded home equipment providers. No study-related procedures or treatments were provided by these representatives. Patients treated themselves at home for 90 min daily five times per week with either the allocated TWO2 or sham therapy. Dressing changes were performed at home by either the patient or their personal caregiver. No study therapy was done at the study centers.

Patients visited a local study center weekly for the duration of the study for wound assessment, debridement, and digital wound photographs. Patients recorded therapy and off-loading compliance daily on diary cards that were verified at each study visit. Additionally, therapy hours were verified by the TWO2 device itself. The active treatment phase was continued until the ulcer healed or for a maximum of 12 weeks.

Data Collection and Outcome Measures

The treatment phase of the study was 12 weeks. The randomization visit measurement after debridement served as the index (baseline) measurement. If multiple ulcers were present, the largest area ulcer at the baseline visit was designated the index ulcer. Weekly digital wound images were transmitted electronically and were assessed for area changes and closure confirmation by a single blinded central assessor using automated CE-marked

Table 1—Inclusion/exclusion criteria

Inclusion criteria	Exclusion criteria
Males and females aged between 18 and 89 years	Evidence of gangrene on any part of affected limb
Documented diagnosis of type 1 or 2 diabetes	Documented evidence of osteomyelitis on any part of affected limb
Foot ulcer at or below ankle with duration >4 weeks to <1 year • If the index ulcer is postamputation, date of surgery must be >30 days • If >1 ulcer is present, largest is considered as the study index ulcer • Index ulcer must be ≥1 cm from any other ulcers present on the foot	Index ulcer has exposed bone Active Charcot foot on the study limb Uncontrolled diabetes: HbA_{1c} >12% (108 mmol/mol) Renal dialysis or creatinine >2.5 mg/dL (221 μmol/L)
Ulcer size ≥1 and ≤20 cm^2 after debridement at start of run-in period	Known immune insufficiency
Ulcer of UTC grade 1A, 1B, 1C, 1D, 2A, 2B, 2C, or 2D	Active treatment for malignancy (not specific to study limb)
ABI >0.7 with a $TcPO_2$ >30 mmHg, skin perfusion >30 mmHg, toe pressure >30 mmHg, or Duplex ultrasound with biphasic waveforms below the knee	Chronic steroid use or immunosuppressive agents within the last 3 months or anticipated to require them during the duration of the study
No planned revascularization procedure or vascular surgery within the last or next 30 days	Subject participated in another investigational device, drug, or biological trial within last 30 days
Subject and caregiver willing and able to comply with all specified care and visit requirements	Index ulcer exhibits signs of severe clinical infection that requires hospitalization or immediate surgical intervention
Subject has a reasonable expectation of completing the study	Subject is pregnant at the time of screening
Subject completed 2-week run-in period with <30% wound size reduction	Subject has had a deep vein thrombosis within the last 30 days Subject has received growth factor therapy, autologous platelet-rich plasma gel, bilayered cell therapy, dermal substitute, extracellular matrix, etc., within the screening period

wound measurement software (MOWA; Healthpath srl, Rome, Italy).

Once a wound was initially determined to be closed by the blinded study site investigator, that visit served as the first of two confirmatory visits. Wound closure (complete epithelialization) was confirmed at the second closure visit 2 weeks later (28). Upon completion of the 12-week treatment phase, patients entered the posttreatment follow-up period for an additional 38 weeks, whereby they returned for wound closure assessment and quality of life (QOL) questionnaires.

The maximum duration for participation in the study was 54 weeks. During the follow-up phase, patients without healed ulcers received standard care according to their clinician's recommendation and were asked not to participate in another wound care trial.

The primary study end point was the percentage of ulcers in each group achieving 100% healing at 12 weeks. Secondary end points included wound area reduction, 12-month incidence of both recurrence and complete healing, incidence of amputation, Cardiff Wound Impact Schedule (CWIS) QOL assessment, and adverse events (1,28,29).

Statistical Analysis

All analyses were performed solely on the ITT study population using Stata 12 (StataCorp, College Station, TX). Results are reported to one decimal place; *P* values and SDs have been reported to two significant figures. For the primary end point of ulcers achieving 100% healing at 12 weeks, statistical significance was assessed at the Pocock 2.2% level ($P < 0.022$). Logistic regression analysis was used to determine the influence of possible confounding variables. Model diagnostics were used to check regression model assumptions and transformations if they did not hold. For this analysis, a backward elimination process was used incorporating the following variables: age, sex, ulcer area, ulcer duration, presence of neuropathy, UTC grade, and HbA_{1c} (%). The same potential confounders were examined within the Cox proportional hazards model. Confounders were included in both models if they changed the odds ratio (OR) or hazard ratio (HR) by >10%. The final logistic regression model and longitudinal hazard models included 97.8% CIs. For all other analyses, statistical significance was assessed at the two-sided 5% level ($P < 0.05$) with 95% CIs provided as appropriate. The statistician conducting all analyses was blinded to treatment allocation (with groups identified as A and B) until results had been finalized.

RESULTS

Between November 2014 and December 2017, 136 patients were screened for the study. Of these, 63 patients (46%) were excluded from randomization for not meeting the inclusion criteria. Thirty-four patients (25%) returned from the 2-week run-in with wound size reductions ≥30%, 10 (7%) had ABI values or second vascular assessments out of range, and 19 (14%) either were not willing to comply fully with the protocol or had other laboratory values out of range. Therefore, 73 patients were randomized into the active phase of the study (see Fig. 1).

At baseline, 65 patients (89%) had type 2 diabetes and 8 patients (11%) had type 1 diabetes. Fourteen index ulcers (39%) in the active arm, compared with six index ulcers (16%) in the sham arm, were assessed to be UTC grade 2 (penetrating to tendon or capsule). Conversely, 22 ulcers (61%) in the active arm, compared with 31 ulcers (84%) in the sham arm, were assessed to be UTC grade 1 wounds ($P = 0.038$). Additionally, 10 patients (28%) in the active arm, compared with 4 patients (11%) in the sham arm, had a previous diagnosis of PAD ($P = 0.066$). Seventeen patients (47%) in the active arm had a history of prior amputations on the index limb in contrast to eight (22%) in the sham arm ($P = 0.018$) (see Table 2).

Primary Outcome

At the first ITT analysis point of 73 patients, the independent data monitoring committee recommended that enrollment should conclude per the predetermined stopping rules, as the active arm

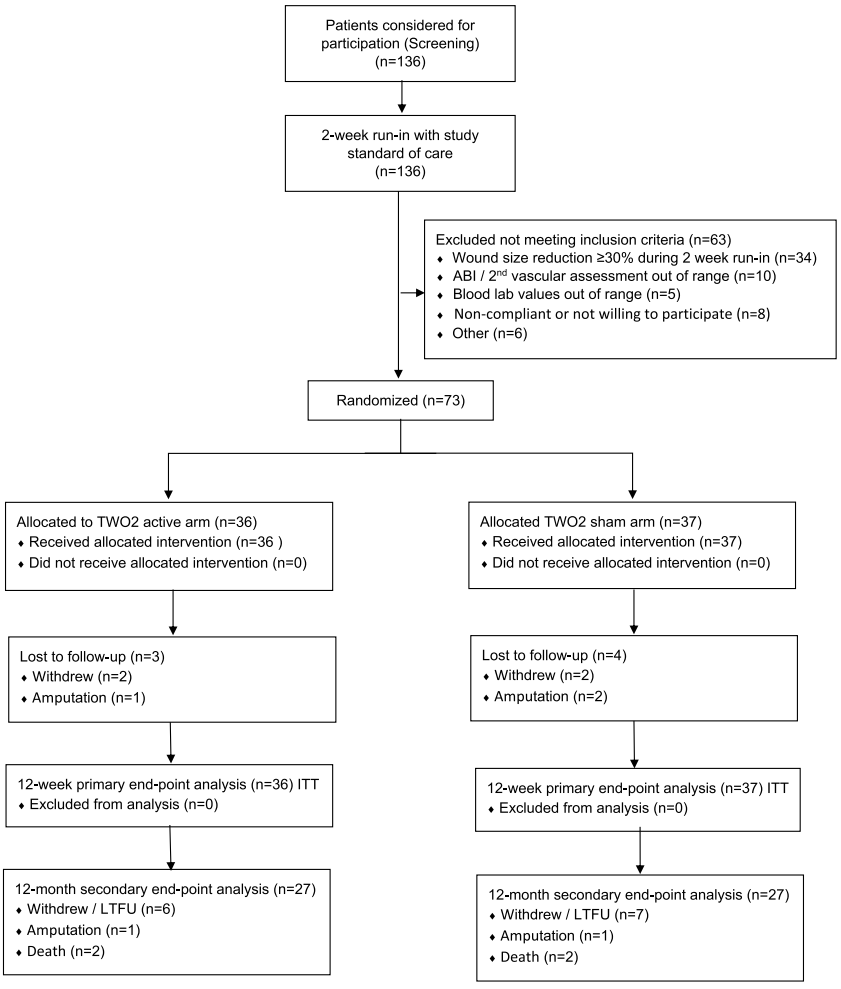

Figure 1—CONSORT diagram of study flow. lab, laboratory; LTFU, lost to follow-up.

Table 2—Baseline characteristics

	Sham TWO2 (n = 37)	Active TWO2 (n = 36)	Total (n = 73)	P
Age, years, mean (SD)	61.9 (9.5)	64.6 (10.3)	63.3 (9.9)	0.21
Sex, male, *n* (%)	31 (84)	32 (89)	63 (86)	0.53
Race, *n* (%)				
White/Hispanic	24 (65)	26 (72)	50 (68.5)	0.90*
Black	5 (14)	5 (14)	10 (14)	
Asian	1 (2.7)	2 (5.6)	3 (4.1)	
American Indian	1 (2.7)	0 (0)	1 (1.4)	
Not reported	6 (16.2)	3 (8.3)	9 (12.3)	
Type 2 diabetes, *n* (%)	33 (89)	32 (89)	65 (89)	0.97
BMI (kg/m^2), mean (SD)	31.2 (7.6)	30.8 (5.9)	31 (6.8)	0.85
Wound area (cm^2), mean (SD)	3.22 (2.54)	3.02 (2.66)	3.13 (2.57)	0.74
Wound perimeter (cm), mean (SD)	6.85 (4.18)	6.22 (2.85)	6.54 (3.55)	0.45
Ulcer duration (days), mean (SD)	174.6 (94)	160.3 (96)	166.4 (95)	0.53
Wound classification, *n* (%)				
UTC grade 1A	27 (73)	20 (56)	47 (64)	
UTC grade 1B	2 (5.4)	1 (2.8)	3 (4.1)	
UTC grade 1C	2 (5.4)	1 (2.8)	3 (4.1)	
UTC grade 2A	4 (10.8)	9 (25)	13 (17.8)	**0.04****
UTC grade 2B	0 (0)	1 (2.8)	1 (1.4)	
UTC grade 2C	2 (5.4)	4 (11.1)	6 (8.2)	
Neuropathic foot, *n* (%)	29 (78)	28 (78)	57 (78)	0.95
Charcot deformity, *n* (%)	3 (8.1)	1 (2.8)	4 (5.4)	0.32
Ulcer location, *n* (%)				0.32
Dorsal foot	5 (13.5)	8 (22.2)	13 (17.8)	
Leg below malleoli	4 (10.8)	1 (2.8)	5 (6.8)	
Pedal foot	22 (59.5)	18 (50)	40 (54.8)	
Toe	6 (16.2)	9 (25)	15 (20.5)	
Previous history of lower-extremity amputation, *n* (%)	8 (21.6)	17 (47.2)	25 (34.3)	**0.02**
Comorbidities, *n* (%)				
Hypertension	30 (81)	28 (78)	58 (79)	0.73
Cardiovascular disease	9 (24.3)	13 (36.1)	22 (30.1)	0.27
PAD	4 (10.8)	10 (27.8)	14 (19.2)	0.07
Venous disease	1 (2.7)	2 (5.6)	3 (4.1)	0.54
Renal disease	6 (16.2)	10 (27.8)	16 (21.9)	0.23
Neurologic disease	31 (83.8)	28 (77.8)	59 (80.8)	0.52
Peripheral edema	1 (2.7)	3 (8.3)	4 (5.4)	0.29
Hyperlipidemia	25 (67.6)	23 (63.9)	48 (65.8)	0.74
Smoker, *n* (%)	10 (27)	13 (36)	23 (31.5)	0.41
Peripheral arterial circulation parameters				
Mean ABI (SD)	1.00 (0.23)	1.07 (0.23)	1.03 (0.23)	0.20
Mean toe systolic blood pressure (SD), mmHg	83.00 (32.75)	84.50 (30.55)	83.77 (30.63)	0.84
Blood work values, mean (SD)				
Prealbumin, μmol/L	4.29 (1.45)	4.44 (0.93)	4.36 (1.18)	0.61
CRP, nmol/L	140 (173)	65.7 (96.2)	99.6 (139)	**0.05**
Creatinine, μmol/L	105.2 (30.1)	113.2 (81.3)	108.7 (61)	0.57
HbA_{1c}, %	8.14 (1.49)	8.43 (1.75)	8.25 (1.64)	0.46
HbA_{1c}, mmol/mol	65 (16.3)	69 (19.1)	67 (17.9)	0.46

All comparisons are nonsignificant except for values in boldface type. *Due to low frequency in each cell, white race was compared with all other races combined. **Due to low frequency in UTC categories, UTC I was compared with UTC II.

was shown to be superior to the sham arm for the primary outcome. In the active arm 15 wounds (41.7%) completely healed versus 5 wounds (13.5%) in the sham arm at 12 weeks [Pearson $\chi^2 = 7.27$ (1 df), $P = 0.007$]. The difference in outcome produced an OR of 4.57 (97.8% CI 1.19, 17.57), $P = 0.010$. Examination of the potential confounding by other baseline variables revealed that UTC ulcer grade substantially changed the OR in favor of the TWO2 group (OR = 6.00 [97.8% CI 1.44, 24.93], $P = 0.004$). The active TWO2 arm showed >3.5 times the likelihood to completely heal over 12 weeks compared with the sham arm with an HR of 3.64 (97.8% CI 1.11, 11.94), $P = 0.013$. With inclusion of the UTC ulcer grade into the model, the HR increased to 4.66 (97.8% CI 1.36, 15.98), $P = 0.004$. The Kaplan-Meier curve shown in Fig. 2 clearly shows the separation between groups throughout the active phase of the study. The patients then entered into the follow-up phase of the study where they were assessed for index ulcer recurrence, healing, and QOL changes for 12 months postenrollment (see Table 3).

Secondary Outcome Measures

Ulcer Recurrence

At 12 months postenrollment, only 1 of 15 healed ulcers (6.7%) in the active arm recurred, compared with 2 of 5 healed ulcers (40%) in the sham arm, falling just short of statistical significance ($P = 0.070$). In total, 20 (56%) active arm (SC+TWO2) ulcers were closed at 12 months postenrollment compared with 10 (27%) of the sham arm (SC+SHAM) ulcers [χ^2 (1 df) = 6.13, $P = 0.013$].

Wound Area Reduction

Of the patients with open ulcers at the end of the 12-week active phase, the mean (SD) absolute reduction in ulcer area from baseline was 1.97 (2.75) cm^2 for the active arm compared with 0.40 (1.75) cm^2 for the sham arm [t (df) = 2.12 (35), $P = 0.041$].

For the patients with larger open ulcers >4 cm^2 at the end of the active phase, the mean (SD) absolute reduction in ulcer area from baseline was 4.12 (1.51) cm^2 for the active arm compared with a 1.34 (1.18) cm^2 increase for the sham arm [t (df) = 2.85 (8), $P = 0.021$].

QOL

The wound care–focused CWIS QOL index improved during the study for patients whose ulcers healed across all functional domains. This positive increase was observed in both full and partial responders. The greatest improvement was seen for the well-being

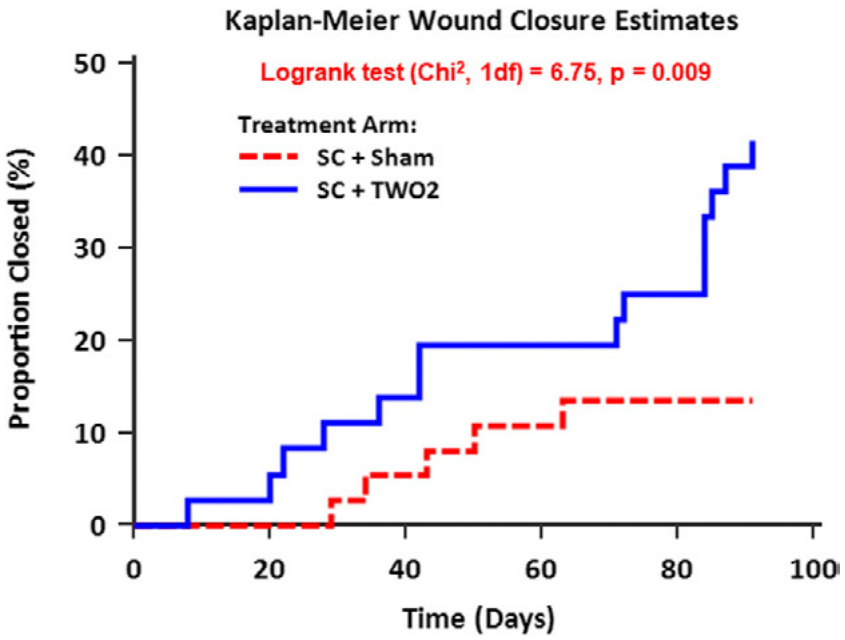

Figure 2—Kaplan-Meier curve showing the separation between study groups throughout the 12-week trial.

component, with mean (SD) score difference between baseline and the end of 12-week treatment in the active arm of 9.1 (13.9) compared with −0.1 (16.9) in the sham arm [t (df) = 2.18 (53), P = 0.033].

TWO2 Therapy and Off-loading Compliance

Therapy compliance in both the active and sham arms was high, with 94% and 96% completing treatments, respectively. Off-loading device compliance in both the active and sham arms was also high, with 97% and 99% using the off-loading >75% of the time.

Adverse Events

During the study, there were equal numbers of serious adverse events (10) and adverse events (8) experienced in both study arms. There were no TWO2 device–related adverse events reported. Two index limb amputations (5%) occurred in the active arm compared with three index limb amputations (8%) in the sham arm.

CONCLUSIONS

TOT has been reported to improve healing of DFUs in several earlier prospective randomized studies (20,27,30,31). However, these studies suffered from methodological weaknesses, such as a lack of blinding, uncontrolled SOC, or inappropriate analyses of the ITT populations. The present TWO2 study has demonstrated, in a randomized, sham-controlled trial, that cyclical pressurized TOT adjunctive to optimal SOC is significantly superior to standard care alone in healing recalcitrant DFUs within a 12-week home-based treatment period. To this end, trial enrollment was terminated at the first predetermined analysis point, since the primary end point had been achieved after the initial 73 randomized patients had completed their 12-week treatment phase.

Despite the loss of 25% of patients in the 2-week run-in period prior to randomization, a four-and-a-half–fold increased likelihood of healing was achieved at 12 weeks in patients allocated to the active TWO2 therapy. With adjustment for UTC ulcer grade, this effect increased even further. A very high degree of compliance with treatment and off-loading was demonstrated in both groups. Clinically, the durability of healing as measured by index ulcer recurrence at 12 months was sixfold better than that in the sham group and that seen in other studies (2). Of interest, and distinct from other topical oxygen studies, this RCT allowed for patients with up to UTC grade 2 ulcers with modest degrees of ischemia. Although not statistically significant, nearly 28% of patients randomized to the active therapy had a prior history of PAD compared with just 10% in the control group. However, despite double-blinded randomization, a significant 47% of active therapy patients had a history of lower-extremity amputations compared with just 22% in the sham arm.

This study is consistent with results reported in several previous studies using topical oxygen in DFU (20,30–32) and venous leg ulcers (33,34), as well as animal studies (23). Several other reviews of this approach have also suggested mechanisms of action and putative benefits of topically applied oxygen in the management of chronic wounds (13,15,24,26). Blackman et al. (20), in a prospective open-label study, examined the clinical efficacy of TWO2 therapy in healing DFU patients in a community wound care clinic. Patients were allocated to topical oxygen or otherwise treated with advanced moist wound therapy. At 12 weeks, 82.4% of the ulcers in the TWO2 therapy arm and 45.5% in the control arm healed completely (P = 0.04). Median time to complete healing was 56 days in the active and 93 days in the control arm (P = 0.013). Another unblinded comparative study investigated the benefits of continuous diffusion of oxygen compared with variable standard care for DFUs (31). Notwithstanding methodological weaknesses, they found significantly faster rates of healing in the topical oxygen group compared with the standard care group and most notably in deeper ulcers. A more recent randomized placebo-controlled trial using a continuous diffusion of oxygen device for only UTC grade 1A ulcers reported a higher proportion of healed DFUs (32.4% vs. 16.7%, P = 0.033) and a faster time to closure (P = 0.015) in the active group at 12 weeks (30). This study was also planned with a group sequential design; however, their interim analysis end point was not met, and their ITT analysis did not include 35% of randomized patients who were subsequently removed from the trial.

Strengths and Limitations

This TWO2 study followed the guidance for wound-healing therapies put forth by the U.S. Food and Drug Administration (28) as well as subsequent publications from leading authorities calling for more robustly designed sham-controlled RCTs

Table 3—Summary of the results: ITT analysis

	Sham TWO2 (n = 37)	Active TWO2 (n = 36)	Pearson χ^2 or OR or HR (97.8% CI), P value
Primary outcome			
Ulcers completely healed at 12 weeks, *n* (%)	5 (13.5)	15 (41.7)	**χ^2 7.27 (1 df), P = 0.007**
By randomized treatment group, univariate			**OR 4.57 (1.19, 17.57), P = 0.010**
			HR 3.64 (1.11, 11.94), P = 0.013
After adjustment for UT grade			**OR 6.00 (1.44, 24.93), P = 0.004**
			HR 4.66 (1.36, 15.98), P = 0.004
Margin of effect/relative performance	**68%/309%**		
Secondary outcomes			
Healing durability			
Ulcer recurrence at 12 months, *n* (%)	2 (40.0)	1 (6.7)	P = 0.070
Ulcers closed at 12 months, *n* (%)	10 (27)	20 (56)	**P = 0.013**
Margin of effect/relative performance	**52%/207%**		
Healing trajectories			
Absolute change in ulcer area over 12 weeks, cm^2	0.40 (1.75)	1.97 (2.75)	**P = 0.041**
Absolute change in ulcer area in ulcers >4 cm^2 over 12 weeks, cm^2	−1.34 (1.18)	4.12 (1.51)	**P = 0.021**
Time to complete wound closure, weeks	6.3 (1.9)	8.2 (4.2)	P = 0.350
QOL			
CWIS well-being improvement between baseline and week 12	−0.1 (16.9)	9.1 (13.9)	**P = 0.033**
CWIS social life improvement between baseline and week 12	4.1 (12.4)	7.9 (16.9)	P = 0.340
CWIS physical symptom improvement between baseline and week 12	4.6 (11.8)	12.1 (23.2)	P = 0.130
Index limb amputations, *n* (%)	3 (8)	2 (5)	P = 0.668
TWO2 therapy and off-loading compliance			
Used TWO2 therapy device 5 days/week, 90 min/day, *n* (%)	35 (96)	34 (94)	P = 0.978
Used off-loading device >75% of the time, *n* (%)	36 (99)	35 (97)	P = 0.984
Safety analysis			
Incidence of serious adverse events, *n*	10	10	P = 0.943
Wound infection	2	3	
Osteomyelitis	5	2	
Hypoglycemic event	1	0	
Urinary tract infection	0	2	
Significant necrotic tissue	1	0	
Cardiovascular event	0	1	
UTC grade 2 ulceration	0	1	
Severe maceration/dermatitis	1	0	
Pneumonia	0	1	
Incidence of adverse events, *n*	8	8	P = 0.950
UTC grade 1 ulceration	0	3	
Ulcer decline	0	2	
Minor infection	1	1	
Minor osteomyelitis	0	1	
Minor necrotic tissue	1	0	
Cellulitis	1	0	
Swelling/edema	1	1	
Maceration	2	0	
Dermatitis	1	0	
Contusion	1	0	
Incidence of adverse device events	0	0	

Data are means (SD) unless otherwise indicated. Boldface type indicates significant differences.

(1,29,35). Nonetheless, and despite randomization of known and unknown potential confounders between groups, it does have limitations. One is the relatively small number of patients included in the primary end point analysis of our ITT population, although the group was similar in size to those of other wound care RCTs (2,36). In a group sequential design study, predetermined hard stopping rules are put in place that in our case were met at the first analysis point of 73 patients. At that point, the primary outcome was achieved by finding significantly more patients in the active group had healed compared with the sham-treated group (41.7% vs. 13.5%, P = 0.007). This approach is used when the magnitude of the treatment effect is uncertain, as it allows for stopping a trial once a wide treatment effect is proven. This also ethically ensures that patients are not further randomized to an inferior arm. In our study, a large margin of effect (68%) and relative performance ratio (309%) were achieved.

The quality of DFU studies is often measured by the results obtained in the control groups. In our sham-treated control group, 13.5% of patients achieved complete ulcer healing within the 12-week outcome period. This rate is similar to that of some studies and lower than

others (17,30,37,38). Interestingly, a recent topical oxygen RCT reported an active group healing rate lower than ours at 32.4% and a similar control healing rate (30). For the more chronic ulcers, their placebo arm healing rate dropped to 13.2%. Despite the large margin of effect between our active and sham groups, we attribute our ostensibly low sham healing rate to the chronicity of the ulcers, complexity of the patients, and the control of, rather than a failure of, SOC treatment. In this regard, the average duration of ulcers enrolled in the trial was >5 months, with a nonsignificant 14-day longer duration in the control group. After the 2-week run-in period, 25% of enrolled patients were excluded from randomization due to a reduction in wound area ≥30%. The study off-loading device, itself proven to be as efficacious as gold standard total contact casting (39), may have enabled progress toward healing that excluded patients likely to heal with such standard care alone. This allowed only patients with wounds more difficult to heal (true SOC failures) to be randomized into this trial. Since there was a very high degree of compliance with both blinded treatments and off-loading throughout the study, we have no reason to believe that the control group healing result was due to any shortcoming in the SOC protocol.

Our sham therapy itself provided nothing more than nonpressurized room air that was free to circulate within the extremity chamber. Room air cannot conceivably be detrimental to the control patients or have a negative impact on ability to heal. Even at the 12-month follow-up evaluation point, long after the active therapy had ended, there was still a clear separation between study groups, with the sham control patients achieving a healing rate of only 27%. Analysis for predictors of healing at 12 weeks resulted only in the treatment effect and UTC ulcer grade being significant. Furthermore, we found no difference in compliance with the therapy or off-loading between study groups. In the absence of otherwise explanatory data to account for the control healing rate, we are left with our presumption that those randomized into the study had ulcers that were truly hard to heal and that the difference in healing rates between active and sham groups was indeed a treatment effect.

The mean age of our study population was ~63 years old, which mirrors that seen in other DFU studies. Eighty-six percent of our study patients were men, likely resulting somewhat from the fact that one-half of the U.S. study sites were Veterans Affairs wound care clinics. Multiple studies have shown DFUs to be more prevalent in men than women to a degree similar to that seen in this RCT (4,10,38). With no significant differences in covariates seen between the two study groups, our findings support the premise that these results are generalizable to similarly afflicted patient populations.

Conclusion

The results of the TWO2 study demonstrate that cyclical pressurized TOT in conjunction with both optimal off-loading and good standard wound care can heal significantly more DFUs at 12 weeks compared with optimal SOC alone. In fact, we found a >4.5-fold increased likelihood of healing within this time period for our actively treated patients. This therapy was safe, without complications, and provided more durable healing for those who had wound closure during active treatment. Uniquely, the therapy has additional benefit in that it can be administered by the patient at home without the expense and difficulties of daily travel to a specialized center. In contrast to recently reported systemic HBOT studies (16,18,40), this robust double-blinded, sham-controlled trial provides evidence to support use of this adjunctive cyclical pressurized TOT for chronic DFUs.

Acknowledgments. The authors thank all study patients, clinic personnel, study coordinators, and colleagues who assisted with patient referrals.

Duality of Interest. This study was sponsored by AOTI Ltd. (Galway, Ireland). The sponsor incurred all costs for the study including all institutional fees, monitoring, data warehousing, statistical services, and the provision of study devices and supplies. R.G.F. received research support from the sponsor during the conduct of the study while employed at the Phoenix VA Healthcare System and has received subsequent speaking honoraria. P.J.F., M.E., J.N.B., L.T., T.W., M.G.G., A.M.L., J.A.T., G.R., C.R.D., K.L., D.G., and S.C.R. all [illegible] research support from the sponsor during the study. No other potential conflicts of interest relevant to this article were reported.

Aside from delivery and home setup of study devices, no sponsor employee or agent participated in any aspect of patient care or study treatments.

Author Contributions. R.G.F. assisted with the conception, design, and analysis of the study and wrote the manuscript. P.J.F. provided the statistical design, performed the analyses, and assisted with writing the manuscript. M.E., J.N.B., L.T., T.W., M.G.G., A.M.L., J.A.T., G.R., C.R.D., K.L., D.G., and S.C.R. contributed to the discussion and critically reviewed and provided edits to the manuscript. R.G.F. and P.J.F. are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Prior Presentation. Parts of this study were presented as a late-breaking abstract at the 78th Scientific Sessions of the American Diabetes Association, Orlando, FL, 22–26 June 2018.

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Vascular and Endovascular Surgery
00(0) 1-8

DOI: 10.1177/1538574412467684
http://ves.sagepub.com
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Technical and Clinical Outcome of Topical Wound Oxygen in Comparison to Conventional Compression Dressings in the Management of Refractory Nonhealing Venous Ulcers

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Abstract

Topical wound oxygen (TWO_2) proposes an option in the management of refractory nonhealing venous ulcers (RVUs). End points are proportion of ulcers healed at 12 weeks, recurrence rates, reduction in ulcer size, and time to full healing. A total of 67 patients with RVU were managed using TWO_2 and 65 patients with conventional compression dressings (CCDs) for 12 weeks or till full healing. Mean reduction in ulcer surface area at 12 weeks was 96% in patients managed with TWO_2 and 61% in patients managed with CCD. At 12 weeks, 76% of the TWO_2-managed ulcers had completely healed, compared to 46% of the CCD-managed ulcers ($P < .0001$). Median time to full healing was 57 days in patients managed with TWO_2 and 107 days in patients managed with CCD ($P < .0001$). After 36 months follow-up, 14 of the 30 healed CCD ulcers showed recurrence compared to 3 of the 51 TWO_2-healed ulcers. The TWO_2 is effective and valuable in managing RVU. The TWO_2 slashes the time required for RVU healing and radically decreases the recurrence rates.

Keywords

topical wound oxygen, venous ulcer, compression dressing

Introduction

Chronic venous ulceration is a common disease. The prevalence is 1% of the total population,[1-4] with 20% of venous ulcers portrayed in octogenarians.[4,5] Ambulatory venous hypertension is the trigger of chronic reperfusion injury. This provokes venous ulceration[1] with its saga of chronicity and recurrence.[1]

Management of venous ulcers costs upward of 1 billion dollars annually in the United States,[6] and around 600 million Euros per year, in a population of 60 million.[7,8] Despite this, recurrence rates have been reported up to 70% in most published series.[9,10]

Over the past 40 years, we learnt that compression will improve the perfusion and ameliorate healing.[2,11,12] Nevertheless, active healthy granulation takes up to 3 weeks to cultivate.[13] The crucial step is how can we speed up the epithelial coverage of a granulating wound?

One therapy that aims at expediting wound healing is topical wound oxygen (TWO_2). Delivered through a Hyper-Box, it promotes angiogenesis and expedites epithelialization. This leads to a higher tensile strength collagen which diminishes scarring and the risk of recurrence.[14-17] It increases the expression of angiogenesis-related growth factors[18,19] and promotes leukocyte function with enhanced bactericidal activity.[20-25]

Aim and Objectives

We aim to assess the technical and clinical outcome of using TWO_2 and conventional compression dressings (CCDs) in chronic refractory venous ulceration (RVU).

We previously published our experience in the use of TWO_2 in chronic RVU.[26] In this current study, we aimed to examine the mid-term efficacy of TWO_2 in managing RVUs and the recurrence rates, after a 5-year follow-up.

Primary end points were proportion of ulcers healed at 12 weeks and recurrence rates at 36 months. Secondary end points were reduction in the ulcer size at 12 weeks, time taken for full

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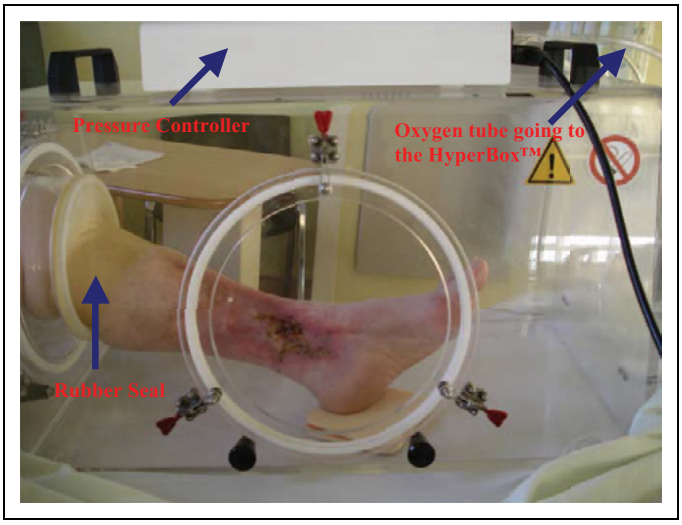

Figure 1. Limb in AOTI-HyperBox. Patient with a medial maleolar ulcer during a TWO_2 treatment session, with the limb placed inside the AOTI-HyperBox. Oxygen and pressure seal is maintained by the rubber cuff, placed below the knee. TWO_2 indicates topical wound oxygen.

healing, and methicillin-resistant *Staphylococcus aureus* (MRSA) elimination.

Inclusion Criteria

A written informed consent was obtained from men/women of age $\geq$18 years.

The duration of the venous ulcer must be more than 2 years with no improvement over the past 1 year in a dedicated veins unit with $C_{6,s}$ in the Clinical, Etiological, Anatomical, and Pathophysiological (CEAP) classification.[27,28] The patient must have a normal ankle-brachial index (ABI) with normal digital pressure.

Exclusion Criteria

Bedridden patients and patients with ischemic ulcers or osteomyelitis in the treated limb were primarily excluded. Patients diagnosed with malignant ulcers were excluded. Diabetes was not considered an exclusion criterion; however, patients with ischemic diabetic ulcers were excluded. A prior pivotal study in our center had proved that the AOTI Hyper-Box (AOTI Ltd, Galway, Ireland) does not work in ischemic diabetic ulcers and might induce iatrogenic deterioration of the affected diabetic limb because of the cyclic pressure.[29,30]

Methods

Study Design

From October 2006 to December 2011, ethical endorsement was attained from patients with chronic RVUs of more than 2 years duration. All patients had to have experienced no sign of progress of the ulcer over the past year, despite ample compliance with appropriate treatment, provided by community-based leg ulcer clinics.

All patients were managed in an intention to treat basis, with the option to be managed either using CCD or using TWO_2. Patients were fully instructed on both the therapies and treatment was conversed with their primary care physician and local tissue viability nurse. Allotment to treatment was centered on the patient's preference.

Techniques

Patients were assessed regarding the anatomical location and the duration of the ulcer, signs of infection, slough, and cellulitis. All vascular risk factors were observed.

The leg ulcer was swabbed for culture and sensitivity. The pain numerical rating scale was used prior to therapy and repeated every 3 days.

Ulcers were cleaned, debrided, digitally photographed, and measured using a Visitrak system (Smith & Nephew Ltd, Hull, United Kingdom), to ascertain the surface area and maximum length and width of the ulcer. Venous duplex ultrasound scan was performed for full CEAP assessment.[27,28] The ABI with big toe digital pressure measurement and punch biopsy were performed for all patients. Patients were assessed regarding their Venous Clinical Severity Score.[31,32]

TWO_2 therapy: 67 ulcers. The limb was placed in the AOTI Hyper-Box for 180 minutes twice daily under pressure of 50 mbars, with oxygen supplied at 10 L/min with continuous humidification (Figure 1). Wounds were washed and left exposed between sessions with no dressings and no compression. Wounds were cleaned, debrided, and remeasured twice per week.[26,29,30]

Compression therapy: 65 ulcers. Full compression was performed, using Profore◊ multilayer compression bandage system with underlying nonadherent Profore◊ wound contact layer dressings (Profore◊ by Smith & Nephew Ltd). Dressings were applied by a wound care specialist nurse and changed as required, 1 to 3 times per week, depending on the amount of exudates.

Protocol Post "Venous Ulcer Healing" or "Failure to Heal"

Treatment was sustained until complete ulcer healing or for 12 weeks, whichever sooner. In either arm of the study, as soon as the ulcer heals the leg is fitted with class 3, closed toe, below knee elastic stockings during the day[33] and advised to rejuvenate the skin of their legs with tap water soaking, baby oil, or olive oil to prevent itching and dry cracked skin with subsequent scratching.

Patients who did not reach complete ulcer healing by 12 weeks, in either treatment arm, were deemed failures of treatment. They were managed with CCD and continued to be seen on a weekly basis. Patients were followed up at 3 monthly intervals following cessation of the therapy.

Table 1. Demographics[a]

Demographics	TWO_2	CCD	*P* Value
Number of ulcers	67	65	
Age (mean/range)	69.34 years (range = 46-85 years)	67.78 years (range = 44-88 years)	.693[b]
Gender, M: F	38: 29	35: 30	.447[c]
Diabetes mellitus	n = 21	n = 18	.425[c]
Smoking	n = 5	n = 2	.628[c]
Hypertension	n = 30	n = 31	.554[c]
MRSA positive	n = 24	n = 19	.291[c]
Patient referred for primary amputation	n = 3	n = 0	.386[c]

Abbreviations: CCD, conventional compression dressings; F, female; M, male; MRSA, methicillin-resistant *Staphylococcus aureus*; TWO_2, topical wound oxygen.
[a] There was no significant difference between both the groups in the demographics or vascular-related risk factors.
[b] *P* value is analyzed using *t* test
[c] *P* values are analyzed using chi-squared test.

Table 2. Characteristics of the Leg Ulcers[a]

Anatomical Distribution	TWO_2, n	CCD, n	*P* Value
Medial maleolus	32	30	.406[b]
Lateral maleolus	16	17	.574[b]
Calf	9	9	.840[b]
Shin	10	9	.801[b]
Ulcer surface area			
$\leq$5 cm^2	9	8	.459[b]
6 to 10 cm^2	10	9	.801[b]
11 to 20 cm^2	25	28	.538[b]
21 to 40 cm^2	12	11	.794[b]
$\geq$41 cm^2	11	9	.715[b]
Duration of the ulcer			
2 to 3 years	12	11	.794[b]
4 to 5 years	23	18	.407[b]
6 to 10 years	19	22	.446[b]
11 to 20 years	9	11	.726[b]
Over 20 years	4	3	.874[b]

Abbreviations: CCD, conventional compression dressings; TWO_2, topical wound oxygen.
[a] There was no statistically significant difference between both treatment groups, regarding the anatomical location of the ulcer, the size of the ulcer, or the duration the patient had the ulcer.
[b] *P* values are analyzed using chi-squared test.

Table 3. The CEAP Classification[a]

CEAP Class[b]	TWO_2, n	CCD, n	*P* Value
$C_{6,s}$	67	65	
E_p	47	51	.186[c]
E_s	20	14	.589[c]
A_s	15	20	.531[c]
A_p	11	7	.769[c]
$A_{s,\ p}$	41	38	.259[c]
P_r	46	42	.217[c]
P_o	4	3	.862[c]
$P_{r,o}$	17	20	.618[c]

Abbreviations: CCD, conventional compression dressings; CEAP class, Clinical, Etiological, Anatomical, and Pathophysiological classification; TWO_2, topical wound oxygen.
[a] There was no significant difference between both the groups in the CEAP classification.
[b] Basic CEAP Classification.[26]
[c] *P* values are analyzed using chi-squared test.

End points were assessed at 12 weeks, apart from the time to full ulcer healing which continued to be assessed beyond the 12 week point. Recurrence rates and quality-adjusted time without symptoms of disease or toxicity of treatment were assessed throughout the treatment and follow-up period.

Statistical Analysis

Data were accumulated and analyzed using SPSS 18 software (SPSS Inc, Chicago, Illinois). Continuous variables were balanced with the independent sample *t* test. Categoric proportions were judged using the chi-squared test. Mann Whitney *U* test was used to compare unpaired, nonparametric data. Time to healing was gauged using Kaplan-Meier with log-rank comparison.

Results

Patients

Over a period of 5 years, from October 2006 to December 2011, 1460 patients were reviewed with a diagnosis of chronic venous ulcers, at our tertiary referral leg ulcer clinic. Of these patients, 431 met the inclusion criteria to be enrolled in this study. After application of the exclusion criteria, only 148 patients were eligible. Out of these, 132 patients consented to join the study.

Totally, 67 limbs with 67 ulcers were managed using the TWO_2 therapy; 65 limbs with 65 ulcers were managed using CCD. In all, 57% of the patients managed with TWO_2 were males (n = 38) and 54% of the patients managed with CCD were males (n = 35; *P* = .447; Table 1).

Risk factors were similar in both the treatment groups (Table 1). There was no significant difference between both the groups in the anatomical distribution of ulcers, size of the ulcers, or the duration of the ulcer (Table 2).

Of the 67 ulcers, 24 ulcers were MRSA positive in the TWO_2 group, while 19 of 65 were MRSA positive in the CCD

Table 4. Previous Ulcer Treatment[a]

Previous Treatment	TWO_2, n	CCD, n	*P* Value
SFJ ligation and division (±perforator avulsion)	7	5	.596[b]
SFJ ligation, division, and LSV stripping (±perforator avulsion)	26	23	.213[b]
SPJ ligation and division (±perforator avulsion)	9	10	.472[b]
Multilayer compression dressings	45	37	.175[b]
Local dressing + elastic stocking	13	18	.286[b]
Local dressing + no compression	9	10	.472[b]

Abbreviations: SFJ, sapheno-femoral junction; LSV, long saphenous vein; SPJ, sapheno-popliteal junction; CCD, conventional compression dressings; TWO_2, topical wound oxygen.
[a] There was no significant difference between both groups regarding the surgical or local treatment the patients had received prior to the study.
[b] *P* values are analyzed using chi-squared test.

Table 5. Results[a]

Results	TWO_2	CCD	*P* Value
Ulcers showing signs of healing in 3 weeks	86% (n = 58/67)	72% (n = 47/65)	.021[b]
Ulcers completely healed by 3 months	76% (n = 51/67)	46% (n = 30/65)	<.0001[b]
Median time to full healing	57 days	107 days	<.0001[c]
MRSA elimination	11/24	0/19	<.001[b]

Abbreviations: CCD, conventional compression dressings; MRSA, methicillin-resistant *Staphylococcus aureus*; TWO_2, topical wound oxygen.
[a] Topical wound oxygen ulcers had a significantly shorter healing rate and healing time, as well as improved methicillin-resistant *Staphylococcus aureus* elimination, compared to conventional compression dressings managed ulcers.
[b] *P* values are analyzed using chi-squared test.
[c] *P* value is log rank.

group ($P = .386$; Table 1).Using the CEAP classification all patients were classified as $C_{6,s}$[27,28] (Table 3).Using the Venous Clinical Severity Score,[31,32] mean score in patients managed with TWO_2 was 25 and was 23 in patients managed with CCD.

There was no significant difference in the previous surgical or local management the patient had received to the ulcers, prior to the study (Table 4).

End points

In all, 86% of the TWO_2-managed ulcers showed a reduction in surface area by 3 weeks of treatment (n = 58/67), compared to 72% of the CCD ulcers (n = 47/65; $P = .021$; Table 5).

The proportion of ulcers completely healed by 12 weeks was 76% in the TWO_2 group (n = 51/67) in contrast to 46% of the CCD group (n = 30/65; $P < .0001$; Table 5).

The mean reduction in ulcer surface area at 12 weeks was 96% in the TWO_2 therapy group, compared to 61% in the CCD group (Figure 2).

The median time to full ulcer healing was 57 days in the TWO_2 group, in contrast to 107 days in the Profore◊ group ($P < .0001$; Table 5; Figure 3).

Within the TWO_2 group, the duration the patient had the ulcer and the size of the ulcer did not affect the healing time. The TWO_2-managed ulcers had a substantially shorter healing time, compared to CCD ulcers, no matter what was the duration of ulcer ($P < .0001$) or the size of the ulcer ($P < .0001$; Table 6). Figures 4 and 5 show an ulcer with a large surface area that healed completely over 8 weeks using TWO_2.

In all, 3 of the patients managed with TWO_2 were referred to our facility for primary amputation following the failure of other treatment modalities, including skin grafting. These 3 ulcers fully healed and none of these patients compelled to have an amputation.

Of the 67 ulcers, 51 of the TWO_2-treated ulcers showed a reverse gradient of healing, where healing commenced from the core of the ulcer and expanded toward the margin (Figure 6). This is conflicting to the conventional healing process that initiates from the outward edges of the ulcer inwardly.

Using the pain numerical ranking scale, the pain score threshold in the TWO_2-managed patients recuperated from 8 to 3 by 13 days.

A total of 11 of the 24 MRSA-positive ulcers in the TWO_2 therapy group were MRSA negative after 5 weeks of treatment regardless of the closure of the ulcer. None of the 19 MRSA-positive ulcers in the CCD group were MRSA negative by 5 weeks of treatment ($P < .001$; Table 5). No local or systemic complications were encountered in either treatment group.

Patients were followed up for a median of 36 months. During that period, 4 TWO_2-managed patients underwent primary varicose vein surgery, while 7 patients (2 TWO_2 and 5 CCD) underwent redo-varicose vein surgery.

During the follow-up, 3 of the 51 fully healed TWO_2-managed ulcers showed signs of recurrence. In comparison, 14 of the 30 fully healed CCD-managed ulcers showed signs of recurrence. Furthermore, 2 CCD-managed ulcers that had not completely healed showed signs of deterioration and increase in surface area ($P < .0001$).

Discussion

The socioeconomic consequences of management of RVU, merged with high recurrence rates, have encouraged the development of a disruptive technology innovative therapy, as TWO_2 therapy.

The McCollum group from Manchester mentioned that contemporary dressing materials do not sway the healing development and that expenses on these products cannot be vindicated on a clinical ground, as they have no proven efficacy. Moreover, they regret that after 30 years of research there is no data to defend using anything other than a simple, inexpensive, low-adherence dressing under multilayer compression in the management of venous leg ulcers.[13]

In the Venous ULcer Cost-effectiveness of ANtimicrobial dressings (VULCAN) trial, it took 101 days to heal 3 cm ulcers. Moreover, only 86% of the small ulcers that had healed at 1 year had a recurrence rate of 14%.[34] This is by using silver dressings on small ulcers that we rarely witness in a typical tertiary vein unit practice.

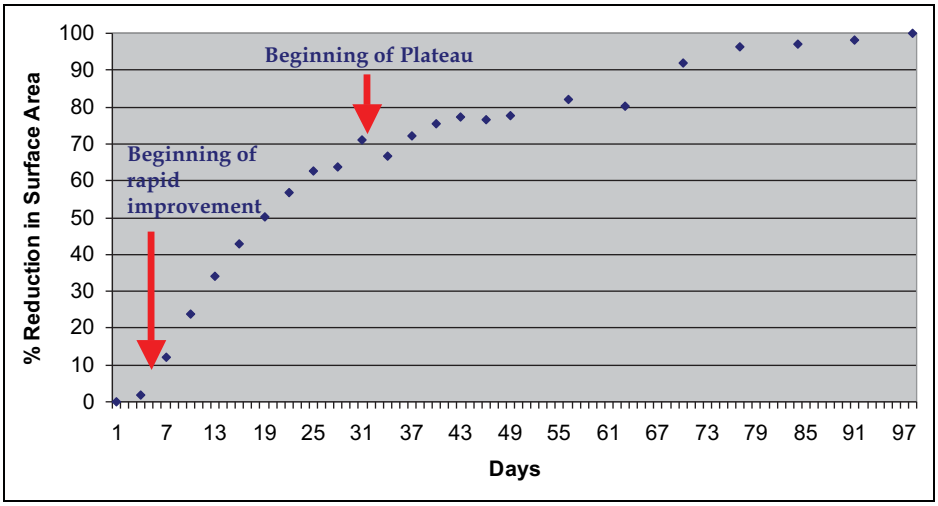

Figure 2. Mean reduction in surface area. There was an initial latent phase up to 5 days, followed by rapid improvement, where the ulcers reached 70% reduction in the surface area. This was followed by a plateau of slow improvement.

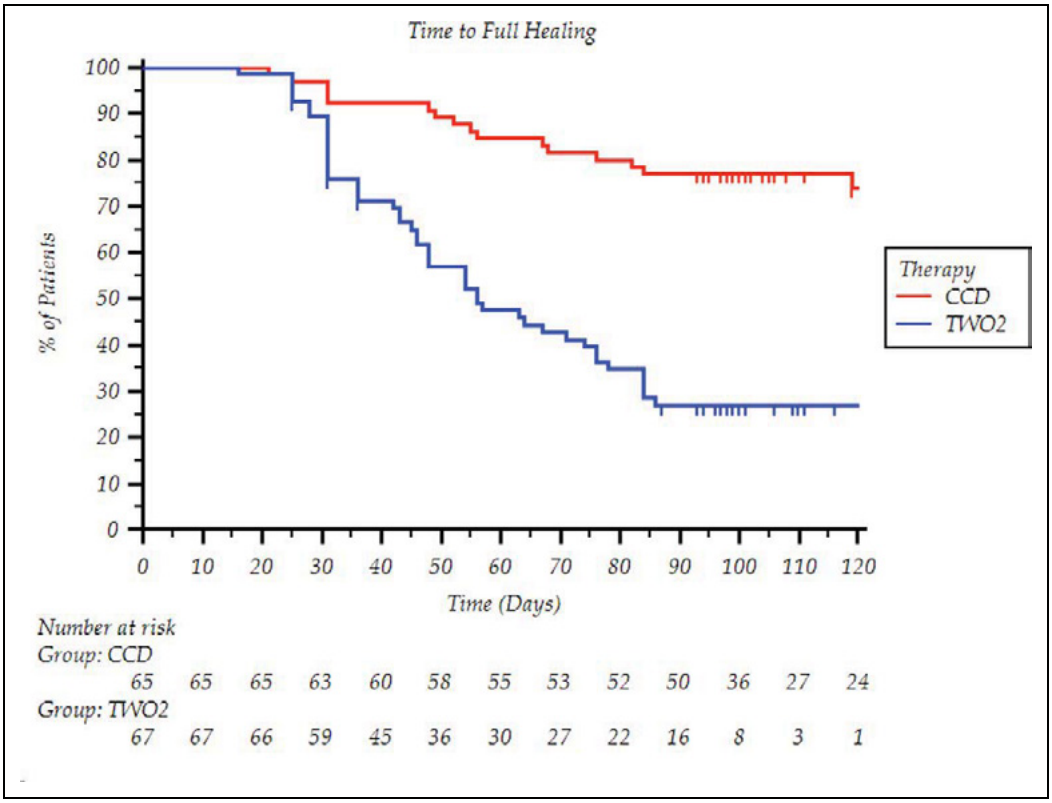

Figure 3. Time to full healing. Kaplan Meier curve showing time to full ulcer healing.
The TWO_2-managed ulcers had a significantly shorter median time to full healing (57days) compared to 107 days in CCD-managed ulcers ($P<.0001$).
TWO_2 indicates topical wound oxygen; CCD, conventional compression dressings.

The TWO_2 circumvents the consequence of a total body hyperbaric chamber, with its drawbacks on eyes, lungs, and ears.[35] Moreover, it eradicates the skyrocket price tag to set up and maintain a total body chamber in a downturn economy, where every Euro and space matters.

The work by Paul Bert verified the toxic consequences of systemic oxygen by yielding grand mal seizures as well as the effort of J. Lorrain-Smith, who confirmed the pulmonary oxygen toxicity, both after systemic administration of oxygen.[35,36] This led to the concept of hyperbaric oxygen

Table 6. Effect of the Size of the Ulcer and the Duration the Patient Had the Ulcer on the Median Duration Required for Healing[a]

Ulcer Surface Area	TWO_2 Median Time to Full Healing	CCD Median Time to Full Healing	*P* Value
≤ 5 cm^2	54 days	87 days	<.0001[b]
6 to 10 cm^2	60 days	118 days	<.0001[b]
11 to 20 cm^2	53 days	109 days	<.0001[b]
21 to 40 cm^2	59 days	113 days	<.0001[b]
≥ 41 cm^2	61 days	119 days	<.0001[b]
Duration of the ulcer			
2 to 3 years	58 days	111 days	<.0001[b]
4 to 5 years	63 days	99 days	<.0001[b]
6 to 10 years	52 days	102 days	<.0001[b]
11 to 20 years	57 days	115 days	<.0001[b]
Over 20 years	59 days	n = 0	<.0001[b]

Abbreviations: CCD, conventional compression dressings; TWO_2, topical wound oxygen.
[a] Topical wound oxygen-managed ulcers had a significantly shorter healing time in comparison to conventional compression dressings, regardless of the size of the ulcer or the length of time the patient had the ulcer.
[b] *P* values are analyzed using Mann Whitney *U* test.

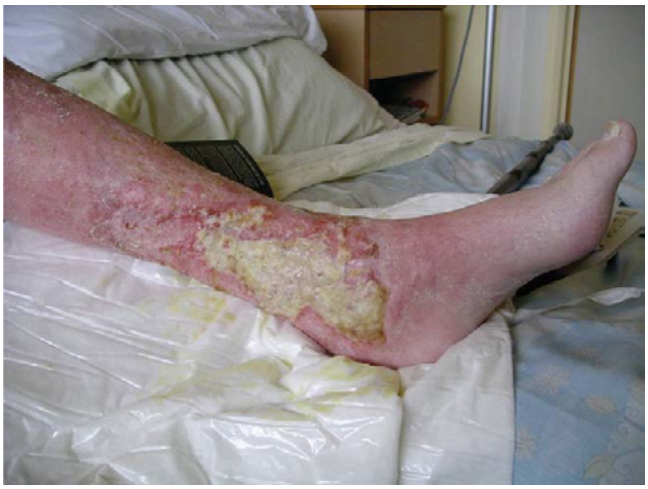

Figure 4. Case 1, Pre-treatment. Large ulcer (98cm^2 surface area) with thick eschar on medial aspect of the leg.

delivery to the site of tissue loss without the side effects of systemic oxygen toxicity.

Conversely, TWO_2 is established on the hypothesis that oxygen diffuses through tissue at a depth of 30 to 50 μm.[36] By calculating all these variables, we established our protocol of cyclic pressure of 50 mbars for 180 minutes twice daily, with oxygen supplied at 10 L/min with continuous humidification.[26,29,30]

The cycling of the pressure in the AOTI Hyper-Box permits the delivery of oxygen under a much higher pressure, allowing improved topical penetration, rather than the limitation of a constant pressure.

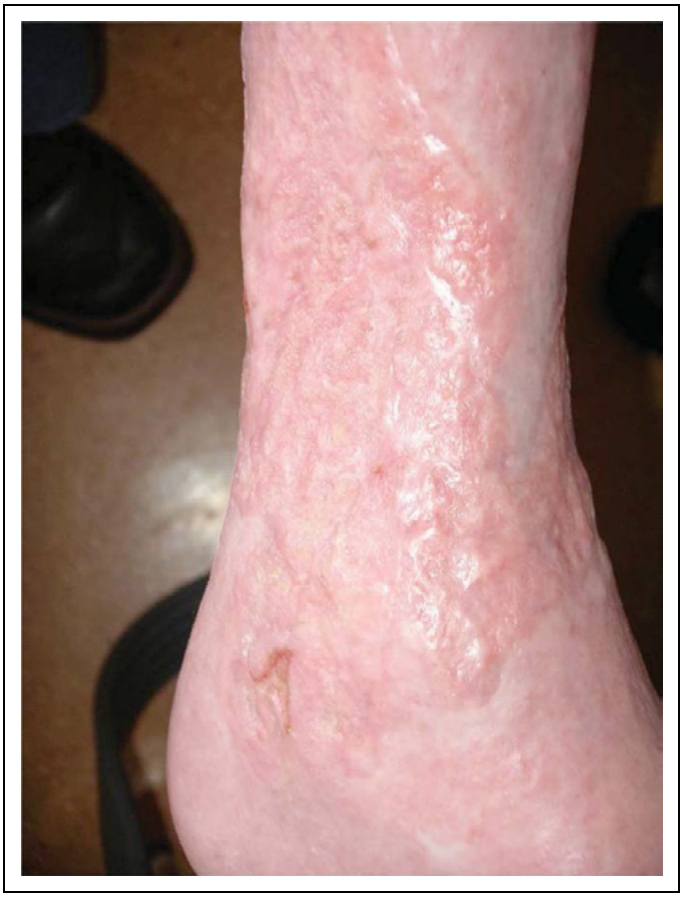

Figure 5. Case 1 after 8 weeks of TWO_2 therapy. Ulcer less than 3cm^2 in the surface area.

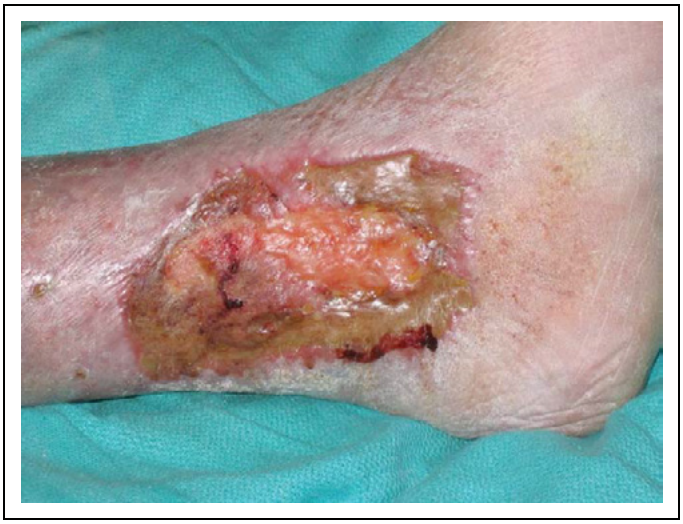

Figure 6. Reverse gradient of healing. Healing starts at the center of the ulcer and then spreads outward.

The TWO_2 promotes capillary neoangiogenesis[18,19] through transdermal sustained delivery of oxygen. This leads to higher tensile strength collagen being formed during wound healing, which eliminates scarring and the risk of recurrence.[14,15]

Diffused oxygen raises the capillary Po_2 levels at the wound site, stimulates epithelization, and granulation of new healthy tissue.[16,17] Repeated treatment accelerates wound closure.

Moreover, oxygen generates reactive oxygen species at the wound site, acting as signaling substances, which increase the production of vascular endothelial growth factor (VEGF).[37,38]

Of the 24 MRSA-positive ulcers in the TWO_2 group, 11 were rendered MRSA negative at the end of their treatment protocol in comparison to none in the CCD group, which outlines the topical bactericidal effect on one of the most feared bacterial infection in the patient's mentality.

The TWO_2 is lethal to anaerobic bacteria and enhances polymorph nuclear function and bacterial clearance.[20-22] It diminishes neutrophil adherence based on inhibition of β-2 integrin function.[23] This enlightens us of its potency against MRSA infection. The TWO_2 assists antibiotic dispersion for aminoglycosides, cephalosporins, quinilones, and amphotericin.[24,25]

Although TWO_2 has been employed over a protracted period of time, the clinical evidence for efficacy and safety are sparse. In our study, we exploited the AOTI Hyper-Box cycled pressure from atmospheric to 50 mbars and back to atmospheric pressure in 1-minute cycles. This permitted the extended treatment administration time while plummeting the risk of endothelial cell toxicity. Our course of therapy accomplished enhanced wound healing time, without complications, in a relatively large number of patients.

During TWO_2 therapy sessions, patients endured limb elevation. These patients had their ulcers for a minimum of 2 years and up to 43 years, and had already revealed no signs of healing over the past year, regardless of ample compliance with the therapy. Although we acknowledge that this may have aided in ulcer healing, it would be futile to accredit the superior outcome to limb elevation alone.

In our study, only 46% of the ulcers managed with CCD fully healed. Although acknowledging that this is a lesser figure than some published studies on such treatment, nevertheless the refractory nature of these ulcers has to be taken into consideration.

In our study 76% (51 of 67) of the TWO_2-treated ulcers exhibited reverse gradient of healing. All these ulcers further continued to fully heal with no scarring and zero recurrence. This is accredited to topical absorption of oxygen which leads to the establishment of privileged tensile strength collagen.[14-17]

Notwithstanding that the mean Venous Clinical Severity Score[31,32] was elevated in patients managed with TWO_2, yet a superior outcome was observed, in contrast to patients managed with CCD, in all facets of clinical and technical outcome.

We believe this to be the principal study in the English literature that embodies venous ulcer management through a portable hyperbaric oxygen chamber and judges against the habitual long-established traditional best medical management in the form of CCD.

The numbers recruited are trivial; however, our foremost ambition was to display the null hypotheses of a disruptive innovative technology with mid-term efficacy and safety. A randomized controlled trial is currently underway to further assess the benefits of TWO_2 therapy.

Conclusion

The TWO_2 is prudent, effective, and valuable in managing RVUs without the risks of full body hyperbaric chambers. The TWO_2 slashes the time needed for RVU healing and is successful in pain alleviation, MRSA elimination, and management.

The TWO_2 radically degrades recurrence rates, thus providing an improved quality of life.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) received no financial support for the research, authorship, and/or publication of this article.

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Topical Wound Oxygen Therapy in the Treatment of Severe Diabetic Foot Ulcers: A Prospective Controlled Study

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Abstract

Diabetic foot ulcers (DFU) are common, difficult-to-treat, and prone to complications. A prospective, controlled study was conducted to: 1) examine the clinical efficacy of a pressurized topical oxygen therapy (TWO_2) device in outpatients (N = 28) with severe DFU referred for care to a community wound care clinic and 2) assess ulcer reoccurrence rates after 24 months. Seventeen (17) patients received TWO_2 five times per week (60-minute treatment, pressure cycles between 5 and 50 mb) and 11 selected a silver-containing dressing changed at least twice per week (control). Patient demographics did not differ between treatment groups but wounds in the treatment group were more severe, perhaps as a result of selection bias. Ulcer duration was longer in the treatment (mean 6.1 months, SD 5.8) than in the control group (mean 3.2 months, SD 0.4) and mean baseline wound area was 4.1 cm^2 (SD 4.3) in the treatment and 1.4 cm^2 (SD 0.6) in the control group *(P* = 0.02). Fourteen (14) of 17 ulcers (82.4%) in the treatment group and five of 11 ulcers (45.5%) in the control group healed after a median of 56 and 93 days, respectively (*P* = 0.04). No adverse events were observed and there was no reoccurrence at the ulcer site after 24 months' follow-up in either group. Although the absence of randomization and blinding may have under- or overestimated the treatment effect of either group, the significant differences in treatment outcomes confirm the potential benefits of TWO_2 in the management of difficult-to-heal DFUs. Clinical efficacy and cost-effectiveness studies as well as studies to elucidate the mechanisms of action of TWO_2 are warranted.

Key Words: controlled prospective study, outpatients, diabetic foot ulcer, topical oxygen therapy, silver dressing

Index: *Ostomy Wound Management* 2010;56(6):24–31

Potential Conflicts of Interest: Dr. Frye discloses he is a consultant for AOTI, Ltd., Galway, Ireland.

Foot disorders such as ulceration, infection, and gangrene, along with subsequent amputation, are significant complications of diabetes, the leading causes for diabetes-related hospitalization, and estimated to cost billions of dollars each year.[1,2] Diabetic peripheral wounds are a major risk factor for lower extremity amputation.[3] Approximately 40% to 70% of all lower extremity amputations are performed in patients with diabetes; approximately 100,000 nontraumatic lower-limb amputations were performed in the US among persons with diabetes in 2008.[4] Even superficial diabetic wounds are often difficult to treat and show high rates of complications.[5]

Oxygen (O_2) is essential to wound healing. Local tissue hypoxia, caused by disrupted or compromised vasculature, is a key factor that limits wound healing.[6,7] It is well established that O_2 is vital in the synthesis of collagen, enhancement of fibroblasts, angiogenesis, and leukocyte function.[8-10] O_2 also has key functions in energy metabolism[11,12] and in the inhibition of microbial growth.[13]

Clinical use of O_2 to promote wound healing began in the 1960s with the administration of systemic full body hyperbaric oxygen therapy (HBO) to treat wounds.[13] Today, HBO is usually administered in single- or multiplace chambers utilizing pressures of 2,500 mb and higher. HBO is reimbursed

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by the Center for Medicare and Medicaid Services in the US to treat certain wounds, including diabetic foot ulcers (DFUs) that have failed to heal using standard care. A Cochrane review by Kranke et al[14] demonstrated that in people with foot ulcers due to diabetes, HBO significantly reduced the risk of major amputation and may improve the chance of healing at 1 year. The availability of HBO facilities, contraindications, the need to transfer the patients to the HBO facilities, and the risks of undesired systemic side effects such as barotraumas of the ear or confinement anxiety limit the widespread use of HBO to treat diabetic ulcers on a global basis.[15]

In an effort to address some of these drawbacks, the principle of topical pressurized oxygen administration or topical wound oxygen therapy (TWO_2) was introduced in the late 1960s.[16] The approach of topically oxygenating the wound is quite different from HBO. TWO_2 does not involve pressures as high as in HBO. Additionally, TWO_2 is portable and can be administered in varied care sites, including in the patient's home. A number of published studies,[16-21] including smaller random controlled trials (RCTs) and case series involving patients with diabetic ulcers, venous ulcers, pressure ulcers, and other wounds demonstrates positive outcomes with TWO_2, but the medical community is not commonly familiar with the principle.

The purpose of this prospective, controlled study was to: 1) compare healing rates of chronic DFUs treated with TWO_2 versus DFUs treated with advanced moist dressing therapy and 2) compare DFU recurrence rates after 24 months in both treatment groups.

Methods

Study design, setting, and population. A prospective, controlled study was conducted at a single center, St. Catharines Wound Clinic, St. Catharines, Ontario, Canada. One trained research nurse in this outpatient wound care center screened patients referred for wound care for study eligibility. Because all devices and dressings are registered products in Canada, no IRB approval was obtained. Informed consent of the participating patients was obtained, including the option to opt out at any time. Patients were considered eligible for participation if they met the following criteria: provision of informed consent, at least 18 years of age, an ankle-brachial index (ABI) of at least 0.5 in the affected limb, and diagnosis of a DFU with a grade 2-A or worse according to the University of Texas (UT) Wound Classification System.[22] Patients were ineligible to participate if they had a chronic wound of nondiabetic origin, deep vein thrombosis (DVT), were pregnant or lactating, were receiving palliative care, were known to be nonadherent with therapy, or had a HbA1c above 10%.

The manufacturer of the topical wound oxygen devices, AOTI Ltd (Galway, Ireland), supported the study by providing the medical devices and the oxygen for use during the study.

Study protocol. After obtaining informed consent, a patient history and baseline assessment were obtained by the study nurse. Variables assessed included: ABI; wound duration and location, and size; loss of protective sensation (determined by 10-g monofilament); and HbA1c. All wounds were classified according to the UT classification for diabetic wounds by an advanced wound specialist based on clinical and laboratory data. All wounds were surgically debrided to a bleeding base; the number of debridements was not limited but usually debridements were performed once a week before treatment commenced. All wounds were offloaded with the Active Offloading Walker (Royce Medical, Camarillo, CA).

If a TWO_2 device was available after the initial assessment (there were a total of four devices), the patient was asked to be in the TWO_2 arm. If all TWO_2 devices were occupied at the first visit of the study participant, or the patient refused daily TWO_2 therapies, the patient was assigned to the control group (see Figure 1) and provided an advanced moist wound therapy (AMWT) using a silver-based dressing (Silvercel™, Johnson and Johnson Inc., Somerville, NJ), which is licensed for the treatment of DFUs by Health Canada.

Hyper-Box Topical Wound Oxygen Therapy Systems (AOTI Ltd., Galway, Ireland) were provided by the Canadian distributor (Therapeutic Surface Solutions Inc., Hamilton, Ontario, Canada) for use in the trial. This system is a class II medical device licensed for the treatment of DFUs as well as other wound types by Health Canada. The device also has US Food and Drug Administration (FDA) 510(k) clearance and CE-Mark approval for the same indications. It delivers humidified medical grade O_2 into an extremity chamber in a cyclical manner. This cycle consists of pressurizing the chamber to 50 mb and then venting the O_2 out of the chamber, allowing pressure to reduce toward ambient pressure (5 mb) before re-pressurizing. Treatment consisted of daily 60-minute TWO_2 treatments, conducted Monday through Friday. Saline-soaked gauze dressings, applied following treatment, remained in place until the next scheduled treatment. Both groups received treatment based on current best practice guidelines, as decided in consultation with three participating surgeons. Dressing changes in the control group also were performed in the study center according to the physicians' recommendation

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Key Points

- A prospective controlled study involving 28 outpatients was conducted to compare outcomes of diabetic foot ulcer treatments.
- The proportion of wounds healed and time to healing was good in both treatment groups but significantly better in the topical oxygen (TWO_2) than in the silver dressing group.
- Research to elucidate the mechanisms of action of TWO_2 and randomized controlled clinical efficacy and cost-effectiveness studies are warranted.

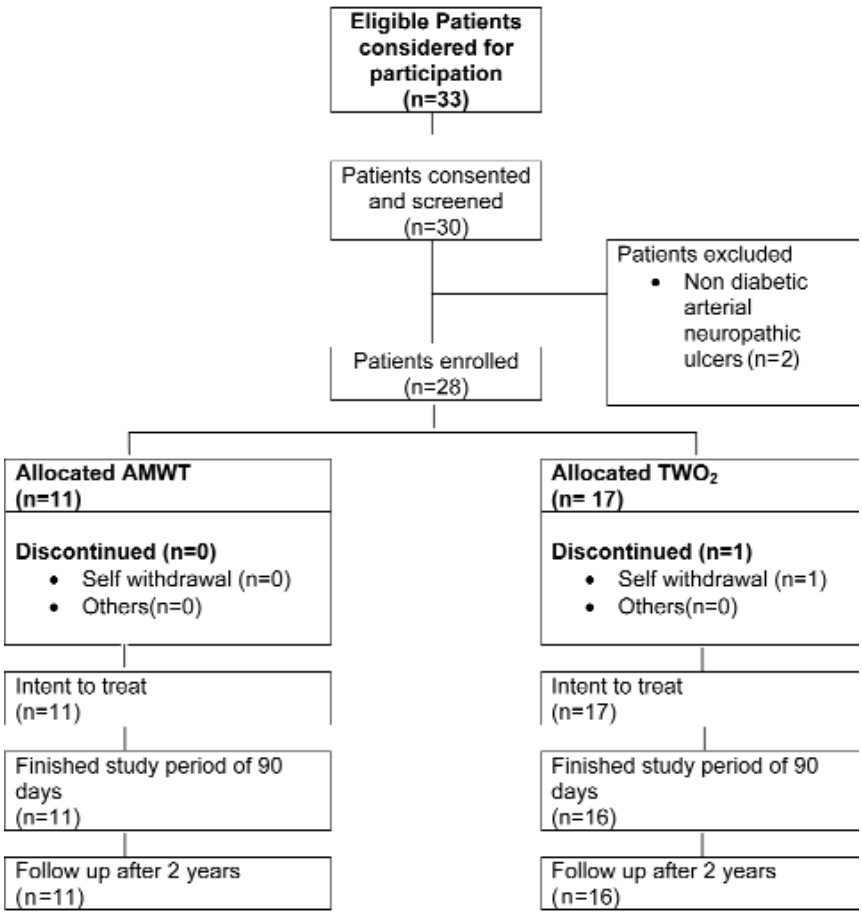

Figure 1. Study population.

at a minimum of twice a week. Each participant's wound was assessed weekly and debrided if necessary. All patients were followed for 90 days in the active treatment phase (ATP) until the wound healed; all patients were monitored monthly for 24 months in the follow-up phases (PUP) to determine if the wound recurred.

The primary study outcome was wound closure, defined as complete epithelialization of the wound with the absence of drainage. The secondary endpoint was reoccurrence rate after 24 months.

Statistical analysis. Data entry was performed twice and computations were performed using the statistical package SAS for Windows version 9.1 (SAS Institute, Cary, NC). Wound area was calculated using length and width measured with a digital caliper. Data from all patients enrolled in the study were analyzed (intent to treat) mainly using a time-to-event strategy with Kaplan-Meier estimates, followed by a log rank test. This statistical procedure provides a comparison of the distribution of events between the two treatment groups. In addition to the event rates, mean and median time to 100% closure were calculated, as well as the proportion of patients with healed ulcers within the active treatment phase. Continuous demographic variables, such as the patient's age at enrollment, were summarized using descriptive statistics and between-group differences were compared with a two-sample *t*-test. Categorical demographic variables such as gender were summarized and compared using a two-tailed chi-square statistic. Comorbidity risk factors were summarized by treatment assignment and according to the type of variable (categorical, continuous) and compared between groups.

Results

In the first week of January 2007, 33 eligible patients were asked to participate in the trial; of these, 30 agreed. Two patients had to be excluded after signing informed consent because they had nondiabetic arterial neuropathic ulcers, leaving a total sample size of 28 patients for follow-up and data analysis. Of those, 27 were followed-up until December 31, 2008 to document DFU reoccurrence in healed wounds. One patient in the TWO_2 group withdrew from the study after 81 days and missing >50% of treatments (see Figure 1).

The TWO_2 and AMWT groups were similar with respect to age, gender distribution, HbA1c, and ABI. Baseline wound area was significantly larger in the TWO_2 than in the control group (mean 4.1 cm^2 [SD 4.3] versus 1.4 cm^2 [SD 0.6]; $P = 0.02$). Wound duration was longer in the TWO_2 group (6.1 months [SD 5.8] versus 3.2 months [SD 0.4] for control) but the difference was not statistically significant. All patients had plantar wounds and peripheral neuropathy as indicated by a loss of protective sensation. No toe or heel ulcers were noted in the study population. Except for one midfoot ulcer in the TWO_2 group, all ulcers were located at the first, third, and fifth metatarsal (see Table 1).

The proportion of ulcers with complete healing was significantly greater in the TWO_2 than in the AMWT group ($P = 0.013$) (see Figure 2). Fourteen (14) out of 17 (82.4%) versus five (5) out of 11 (45.5%), respectively, showed complete epithelialization of the wound ($P = 0.04$). Median time to closure was 56 days (interquartile range [IQR] 39–81 days) in the TWO_2 group and 93 days [IQR: 62–127]) in the control group. In the follow-up phase of up to 24 months, there were no reoccurrences at the healed ulcer site in either the TWO_2 therapy or control group.

No treatment-related adverse events were documented in either group.

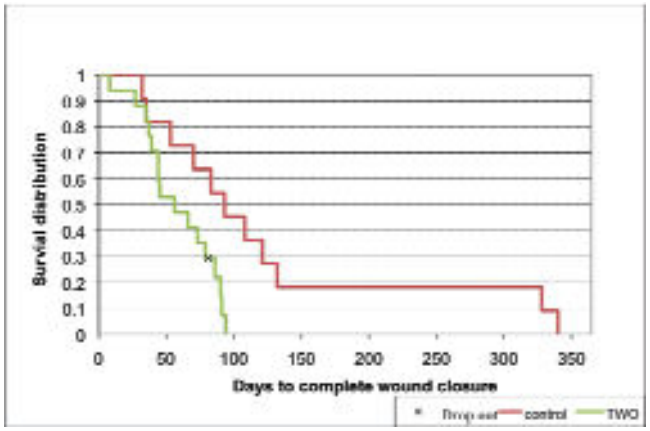

Figure 2. Kaplan-Meier estimate for time to complete wound closure.

Time to complete closure TWO_2 group = 94 days; time to complete closure control group = 340 days (P = 0.013)

Discussion

Overall study results. Wounds in patients treated with TWO_2 in this study were significantly more likely to heal and during a shorter period of time than wounds in patients receiving AMWT. These results must be interpreted within the context of the study design. There was no formal randomization and in the vast majority of cases the secretary of the wound care center assigned the groups based on equipment availability and patient preference without knowledge about wound severity. Nevertheless, all staff members were aware of group assignments and it seems likely that more serious wounds were assigned to the TWO_2 group after noting positive results in a pre-study phase before this study commenced in January 2007. This selection bias helps explain why wounds in the TWO_2 group had a larger surface area, UT classification as more severe, and longer wound duration before enrolling into the study than wounds in the control group. In this respect, the results of this trial may underestimate the potential benefits of TWO_2 compared to AMWT.

On the other hand, it is also possible that a "self-selection" of patients took place in favor of AMWT treatment for persons with less interest in following the protocol of care and visiting the center five times a week. According to the study protocol, patients were given the option not to go into the treatment group but no patient "randomized" by the secretary refused to go into the treatment group.

Patient adherence to protocol (particularly with offloading) in a study of neuropathic DFU is an important factor in healing. All patients received offloading but it is possible that poor adherence is at least partly responsible for the outcome differences observed. An additional potential bias is the positive reinforcement of daily 1- to 2-hour visits for the treatment group versus twice-per-week visits for the control group. Positive reinforcement of weight-bearing limitation is likely to occur during these visits. However, the magnitude of the differences observed is unlikely to have occurred as a result of these potential differences only.

Previous studies[23-27] conducted on DFUs that compare AMWT to other adjunctive modalities have shown proportions of wounds healed ranging from 26% to 46.2% following 12 weeks of care in their control groups. The best results (46.2% healed after 12 weeks) were reported in a prospective, randomized, multicenter study[27] of UT grade 1 or 2 DFUs (n = 86) that investigated healing time between patients receiving a cellular matrix and standard care. The high proportion of wounds healed in the more severe wounds enrolled in the control group of the current study, 45.5% of UT grade 2 and 3 wounds, suggests that the standard of care provided in control group in this wound clinic was good.

The role of oxygen. Although questions about the mechanism of action of TWO_2 remain, evidence suggests that TWO_2 plays a key role in achieving the needed oxygen balance in the wound bed required for wound healing to progress, as suggested by Sibbald and Woo.[28]

It is well established that oxygen is vital in collagen synthesis, fibroblast enhancement, angiogenesis and leukocyte function.[8-10] Hypoxia caused by disrupted vasculature is a key factor that has been found to limit wound healing.[6,7] The partial pressure of oxygen (pO_2) in the wound is lower than in healthy tissue; in dermal wounds, pO_2 ranges from 0 to 10 mm Hg in the center of the wound to 60 mm Hg at the periphery.[6] In contrast, the pO_2 in arterial blood is approximately 100 mm Hg.

Oxygen needed for collagen synthesis proceeds in direct proportion to pO_2 across the entire physiologic range, from 0 to hundreds of mm Hg. Collagen synthesis requires several enzymes. A measure to characterize an enzyme is the substrate concentration at which the reaction rate reaches half of its maximum value (Vmax/2). This concentration can be shown to be equal to the Michaelis constant (KM). The KM of O_2 in collagen synthesis has been determined to occur at a pO_2 of 20 to 25 mm Hg. Vmax is approximately 250 mm Hg, suggesting that new vessels cannot approach their greatest possible rate of growth unless the wound tissue pO_2 is as high as 66.[29] Consequently, *in vivo* and human studies have shown that hypoxic wounds deposit collagen poorly and are more likely to become infected.[30]

Recent research has focused on oxygen and infection. In a wound bed, large amounts of molecular oxygen are partially reduced to form reactive oxygen species (ROS). Leading researchers view the NADP(H)-linked oxygenase as a key factor. *In vitro* studies have shown that this enzyme increases leukocytic oxygen consumption by as much as 50-fold and subsequently uses most of the oxygen delivered to wounds.[31] The NADPH oxidase catalyzes the production of ROS by phagocyte cells such as neutrophilic and eosinophilic granulocytes, monocytes, and macrophages. Exposing these phagocytes to an infectious stimulus activates a "respiratory burst" caused

Table 1. Baseline patient and wound characteristics

Characteristics	Control group N = 11	TWO_2 group N =17
Age (years)	63.4 (9.6)	62.4 (9.7)
Gender (male)	8 (72.7%)	12 (70.6%)
HbA1c (%)	7.4% (1.2%)	7.3 (1.2)
Current smoker	0 (0%)	2 (11.8%)
Ankle-brachial systolic pressure index (mm Hg)	1 (0.18)	0.9 (0.21)
Wound duration before therapy (months)	3.2 (0.4)	6.1 (5.8)
Wound area (cm^2)	1.4 (0.6)[a]	4.1 (4.3)[a]
Wound stage		
C II	0 (0%)	0 (0%)
C III	0 (0%)	1 (5.9%)
D II	7 (63.6%)	5 (29.4%)
D III	4 (36.4%)	11 (64.7%)
Received offloading therapy	11 (100%)	17 (100%)
Plantar location of wound	11 (100%)	17 (100%)
1st metatarsal	10 (91%)	4 (22%)
3rd metatarsal	1 (10%)	1 (6%)
5th metatarsal	- -	11 (61%)
Midfoot	--	1 (5%)
Loss of protective sensation	11 (100%)	17 (100%)
History of plantar ulceration	10 (90%)	15 (88%)
Charcot foot		1 (5.9%)

Data are mean (SD) or number of patients (%)
[a]$P = 0.05$

by activation of the plasma membrane-bound NADPH oxidase. Research presented by Hunt[13] has shown that approximately 98% of the oxygen consumed by wound neutrophils is utilized for respiratory burst. In simpler terms, the majority of oxygen in infected chronic wounds is probably used to fight infection via the ROS-system, leaving almost no oxygen for wound healing.

The ROS includes oxygen-free radicals such as the superoxide anion (O_2-)as well as hydrogen peroxide (H_2O_2). The superoxide anion also drives endothelial cell signaling required during angiogenesis. Endogenous hydrogen peroxide drives redox signaling, a molecular network of signal propagation that supports key aspects of wound healing such as cell migration, proliferation, and angiogenesis.[32]

In summary, the dilemma in wound healing is that the oxygen supply is limited while oxygen demand increases significantly. Three major factors are responsible for wound tissue hypoxia: peripheral vascular diseases (PVDs) limiting the blood supply and thus the needed oxygen; increased oxygen demand of the healing tissue needed for collagen synthesis and angiogenesis; and the generation of ROS needed for infection control (respiratory burst) and redox signaling

Topical oxygen therapy. The big question is whether topical oxygen can penetrate the wound surface to increase the pO_2 in the wound tissue. Fries et al[18] studied the efficacy of topical oxygen in an experimental setting using a pre-clinical model involving excisional dermal wounds in pigs. Exposing open dermal wounds to topical oxygen treatment increased superficial wound tissue pO_2. Fries et al used a probe designed to measure superficial pO_2 at 2 mm depth at the center of the wound bed and saw an increase of pO_2 from the baseline of 5 to 7 mm Hg to 40 mm Hg in as little as 4 minutes. More indirect evidence of the oxygen penetration into the tissue with topical oxygen devices comes from Scott and Reeves'[33] uncontrolled experiments on three patients with plantar diabetic wounds. Using multiplex ELISA assays of growth factor cytokines, the authors quantified levels of total proteins detectable in fluids collected twice weekly from wounds after exposure to topical oxygen. TWO_2 was shown to increase the levels of a variety of angiogenesis-related growth factors (BFGF, HB-EGF, KGF and VEG-F) in chronic wounds. In chronic DFUs treated with TWO_2, the most crucial angiogenesis-related growth factor, VEG-F, increased as much as 20-fold.[34]

Gordillo et al[32] analyzed data from two simultaneous nonrandomized studies to test the effects of HBO and topical oxygen therapy. In total, 1,854 patients were screened in outpatient wound clinics for nonrandomized enrollments into the HBO (n = 32; 31% were persons with diabetes) and TWO_2 (n = 25; 52% were persons with diabetes) studies. HBO did not result in statistically significant improvements in wound size or significant changes in the expression levels of any of the genes studied. Topical oxygen treatment significantly reduced wound size and was associated with higher VEGF165 expression in healing wounds.

After an initial prospective case series study by Fisher[16] in 1969, only in the last 5 to 10 years has there been new interest in topical approaches to oxygenate cutaneous wounds.[18-21,28-36] The results obtained in this trial confirm previously published results of using TWO_2 in chronic wounds. In a prospective case series, Fisher[16] treated 52 patients with venous ulcers (n = 16), pressure ulcers (n = 26), and DFUs (n = 2) with topical oxygen that had failed to heal from several months to several years without improvement. The diabetic ulcers were superficial and had been present for 4 and 5 months. With topical oxygen treatment, the two diabetic ulcers healed within 6 and 9 days, failing in six of the 52 cases. In four of these failures, an underlying osteomyelitic process, unknown at the start of therapy, was noted. In the same study, six patients had almost identical lesions on both lower extremities and hips. One lesion was treated conventionally and the contralateral lesion was treated with topical oxygen. Two of six control-treated

wounds showed mild improvement; all TWO_2 treated wounds healed within 7 weeks.

Heng et al[20] conducted a prospective randomized controlled study utilizing TWO_2. Participants included 40 inpatients with 79 necrotic/gangrenous ulcers assigned to TWO_2 or control treatment. The ulcers were of mixed etiology — 39 were diabetic ulcers, 23 of which were located on the foot. Control group patients received standard wound care including sharp debridement as needed and wet-to-dry or hydrocolloid dressings were changed one to three times daily. TWO_2 consisted of topical oxygen delivered at 1.03 to 1.04 atmospheres, with treatment set at 4 hours per day, 4 days per week, for a maximum treatment time of 4 weeks. In the TWO_2 group, 90% of ulcers healed compared with 22% in the control group.

Heng et al[21] also conducted a 3-month prospective cohort study to assess the healing rate and cost-effectiveness of TWO_2 in healing necrotic/gangrenous wounds in patients with and without diabetes. Necrotic tissue was debrided by sharp debridement and infected ulcers were treated with oral or intravenous antibiotics. Gangrenous digits or forefeet were treated by partial amputation with subsequent treatment of the skin defect with TWO_2. Fifteen (15) patients had 24 wounds, out of which 22 healed in 24 weeks.

Tawfick et al[36] recently published the results of an 83-patient parallel observational study comparing TWO_2 and conventional compression therapy used in venous ulcer management. After 12 weeks, 80% of TWO_2-managed ulcers were completely healed (median 45 days) compared to 35% of the control group ulcers (median 182 days) (P <0.0001). Pain scores in TWO_2-managed patients improved and nine of the 19 methicillin-resistant *Staphylococcus aureus* (MRSA)-positive ulcers in the TWO_2 group were MRSA-negative after 5 weeks of treatment regardless of ulcer closure compared to none of the 17 MRSA-positive ulcers in the control group.

Implications for practice. The diabetes epidemic is a worldwide problem. In the most recent national cross-sectional study[37] from the year 2000 of coronary risk factors in Saudi Arabia (the CADIS study), 23.7% of adults over 40 years of age had diabetes. The sample included 16,806 adults and the final response rate was 93%. In 2007, more than 100,000 patients with diabetes in the US had a foot amputation.[4] The mortality rate after a diabetes-related lower leg amputation is high. A retrospective database query and medical record review for January 1, 1990, to December 31, 2001 by Aulivola et al[38] reported survival rates after major amputation of patients with diabetes of 69.7% and 34.7% at 1 and 5 years, respectively. In the current study, the attending orthopedic and vascular surgeons estimated that 25% of the TWO_2 group patients faced imminent risk of amputation had the treatment regimen not been successful.

The financial burden of DFUs is also considerable. An uncomplicated DFU is estimated to cost $8,000 to treat, an infected ulcer can cost $17,000 and the cost of amputation can reach $45,000.[39,40] Considering the results obtained in this and other studies, TWO_2 has the potential to provide substantial cost savings.

Conclusion

A significant difference in the proportion of DFUs healed was observed between daily TWO_2- treated wounds and those managed with advanced wound dressings. TWO_2 is a simple-to-apply, noninvasive therapy. No adverse events were observed in this or previously published studies. During the 24-month follow-up, no reoccurrence of healed ulcers was observed in either treatment group. Well-designed RCTs to confirm the efficacy and evaluate the cost-effectiveness of TWO_2 are needed. n

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Section II

In Vivo Studies

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Dermal excisional wound healing in pigs following treatment with topically applied pure oxygen

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Received 11 February 2005; received in revised form 18 February 2005; accepted 18 February 2005
Available online 18 August 2005

Abstract

Hypoxia, caused by disrupted vasculature and peripheral vasculopathies, is a key factor that limits dermal wound healing. Factors that can increase oxygen delivery to the regional tissue, such as supplemental oxygen, warmth, and sympathetic blockade, can accelerate healing. Clinical experience with adjunctive hyperbaric oxygen therapy (HBOT) in the treatment of chronic wounds have shown that wound hyperoxia may increase granulation tissue formation and accelerate wound contraction and secondary closure. However, HBOT is not applicable to all wound patients and may pose the risk of oxygen toxicity. Thus, the efficacy of topical oxygen treatment in an experimental setting using the pre-clinical model involving excisional dermal wound in pigs was assessed. Exposure of open dermal wounds to topical oxygen treatment increased tissue pO_2 of superficial wound tissue. Repeated treatment accelerated wound closure. Histological studies revealed that the wounds benefited from the treatment. The oxygen treated wounds showed signs of improved angiogenesis and tissue oxygenation. Topically applied pure oxygen has the potential of benefiting some wound types. Further studies testing the potential of topical oxygen in pre-clinical and clinical settings are warranted.

Keywords: Pre-clinical; Therapy; Angiogenesis; Swine

1. Introduction

Hypoxia, caused by disrupted vasculature and peripheral vasculopathies, is a key factor that limits dermal wound healing [1,2]. The pO_2 of dermal wounds ranges from 0 to 10 mmHg centrally to 60 mmHg at the periphery, while the pO_2 in the arterial blood is approximately 100 mmHg. Oxygen delivery is a critical element for the healing of wounds [3–5]. Factors that can increase oxygen delivery to the regional tissue, such as supplemental oxygen, warmth, and sympathetic blockade, can accelerate healing [6,7]. The clinical use of

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doi:10.1016/j.mrfmmm.2005.02.023

oxygen to promote wound healing began in the 1960s with administration of systemic hyperbaric O_2 (HBOT) to treat wounds [8]. Clinical experience with adjunctive HBOT in the treatment of chronic wounds [9] have shown that wound hyperoxia increases wound granulation tissue formation and accelerates wound contraction and secondary closure [10,11]. The application of topical oxygen gas on exposed dermal wounds is also used clinically to oxygenate the wound tissue [2,12–19]. This therapeutic modality remains poorly studied.

While the conditions (e.g., pressure, O_2 concentration, frequency and duration of administration) for systemic hyperbaric O_2 therapy (HBOT) have not been optimized on the basis of randomized clinical trials, HBOT is an FDA-approved therapeutic modality used in wound clinics with variable success. HBOT delivers 100% O_2 at 2–3 atmospheres (atm) of pressure and patients typically receive 10–30 treatments, depending upon the diagnosis. These treatments are usually 60–120 min long, given 5 days a week and performed in specialized chambers at facilities with physician supervision. HBOT is capable of elevating arterial pO_2 as high as 1200 mmHg [2]. This brings with it the clear risk of oxygen toxicity. Like many other risk factors including cigarette smoking, HBOT does not typically result in immediate manifestation of clinical abnormalities. This line of evidence cannot be accepted as proof of safety unless detailed biochemical and molecular investigation is conducted to test markers of oxidative damage in the blood and urine of treated subjects. It is general knowledge that exposure of biological cells and tissues to pure O_2 may result in oxidative stress and genotoxicity [20]. There is no question that exposure to pure O_2 presents risk and that it is prudent to avoid unnecessary exposure to a risk factor. HBOT is contraindicated in a number of clinical conditions. Moreover, some patients opt against HBOT because of claustrophobia as the chambers used to administer HBOT are relatively small.

Favorable outcome in studies using sub-pure O_2 under normobaric conditions [21] lead to question the use of pure O_2 under pressure for wound therapy. Furthermore, encouraging outcome obtained from the use of topical O_2 alone [19] warranted a more detailed investigation testing the efficacy of topical O_2 treatment under controlled conditions. Such fine-tuning of conditions for O_2 therapy should result in a more cost-effective and efficient care minimizing barotraumas and other risks associated with use of pressurized pure O_2. If proven to be efficient, topical O_2 therapy has the added advantage of caring for much larger potential patient population especially under conditions of public disaster and in a field-setting where HBOT may not be applicable. In response to favorable outcomes of the clinical case series study conducted by surgeons at the Ohio State University, we sought to test the efficacy of topical oxygen treatment in an experimental setting using the pre-clinical model [22,23] involving excisional dermal wound in pigs.

2. Materials and methods

Telazol was obtained from Fort Dodge Animal Health, Fort Dodge, Iowa. Telazol (tiletamine HCl and zolazepam HCl) is supplied in individual vials and when this is reconstituted produces a solution containing equivalent of 50 mg tiletamine base, 50 mg zolazepam base and 57.7 mg manitol/ml. Duragesic was obtained from Janssen Pharmaceutica Products, L.P. Titusville, NJ. Duragesic (fentanyl transdermal system; *N*-phenyl-(1-2 phenyl ethyl-4-piperidyl) propanamide) is a transdermal system providing continuous systemic delivery of fentanyl, a potent opioid anaelgesic, for 72 h. Tegaderm bandage was obtained from 3M Health Care, St. Paul, MN. Elastikon (4 in.) bandage wrap material was purchased from Johnson and Johnson, Indianapolis, IN. Punch biopsies were taken using 3 mm dermal punch biopsy supplied by Miltex Inc. York, PA. Topical oxygen devices were provided by GWR Medical, Chadds Ford, PA.

2.1. Experimental model, wounding and treatment protocol

Four female specific pathogen free domestic pigs weighing 80 pound were used. For wounding, the animals were initially sedated using Telazol (tiletamine and zolazepam, 6 mg/kg body weight). During wounding and treatment, animals were kept anesthetized with isofluorane via a face mask. The wound sites over the dorsal trunk area were shaved using a size 40 clipper blade. The area was cleaned using alcohol and Betadine scrub. Excisional dermal wounds ($n = 10$; two sets of 5) were created on the back of each pig using a

size 10 scalpel. A total of 40 wounds in four pigs were studied. Full-thickness sections of skin (1 × 1 in.) were removed during the wounding process. Duragesic (fentanyl transdermal system) patches were placed on the pinna to alleviate pain in response to wounding. All wounds were dressed with a Tegaderm (3 M Health Care, St. Paul, MN) patch. The patches were held in place by a Elasticon bandage wrap (Johnson and Johnson, Indianapolis, IN). After trying several types of bandage material, Elasticon was found to stay adhered to the skin yet it could be easily removed for treatments without irritating the underlying skin. In order to keep the bandages clean, the animals were housed in elevated vinyl-coated wire floored runs. Sterile techniques were utilized when doing bandage changes to minimize introduction of pathogens to the wound site. Finally, the psychological well-being of the pigs was addressed by providing them with conspecific visual interaction, various toys, and hand-fed treats under professional supervision. These forms of enrichment serve to lower the distress that may otherwise be experienced and potentially confound the experimental results.

The Tegaderm dressed wounds were allowed to heal by secondary intention. Half of the wounds were subjected to topical oxygen treatment whereas the other half of the wounds in the same pig was left exposed to room air. Out of five wounds in each treatment group, two were designated for biopsy collection. Punch biopsies (3 mm) were collected from the wound edge at specified time intervals. Animals were provided with standard laboratory diet and water ad lib. Individual housing (70 ± 4 °F; 40–70% humidity) and care for animals were in accordance with the guidelines of the Institutional Lab Animal Care and Use Committee (ILACUC) of the Ohio State University.

For topical treatment with pure oxygen, a plastic device that is routinely used to treat patients was employed [17,19]. The device has a triangular textile base containing skin adhesive. Medical grade oxygen was used to inflate the device and then the flow rate was set to 3–6 l/min. The treatment was performed for 3 h daily for the first 7 days (day 0–6) from the day of wounding.

2.2. Wound area assessment

All wounds were digitally photographed in the presence of a standard reference ruler. Wound area was computed using the WoundMatrix™ software as described previously [24,25].

2.3. Wound-bed pO_2 measurements

Real-time wound-bed pO_2 was performed non-invasively using Oxy-Lite (Oxford-Optronix, Oxford, UK) as described by us previously [17,26]. An O_2 electrode, specially designed for our application purposes by the vendor, was placed at 2 mm depth in the center of the wound bed.

2.4. Histology

Formalin-fixed wound-edges embedded in paraffin were sectioned. The sections (8–10 μm) were deparaffinized and stained with hematoxilin and eosin (H&E) as well as for Masson Trichrome staining for histological analysis using standard procedures [17,26]. Furthermore, the sections were immunostained with the following primary antibodies: Keratin 14 (1:500; Covance, Berkeley, CA), hVEGF (1:50 dilution; R&D Systems, MN) or anti-smooth muscle actin (1:1000; Sigma, St. Louis, MO). To enable fluorescence detection, sections were incubated with appropriate Alexa Fluor® 488 (Molecular probes, Eugene, OR) conjugated secondary antibody (1:250 dilution). In some cases, the sections were stained with DAPI (Molecular probe, Eugene, OR) to visualize the nuclei. Images were collected using a Zeiss Axiovert 200M motorized microscope supported by an AxioCam digital camera, Axiovision software and Apotome.

2.5. Statistics

Data shown as bar graphs are mean ± S.D. Student's paired *t*-test was used to test significance of difference between means. $p < 0.05$ was interpreted as significant difference between means.

3. Results

A clinical topical oxygen device (Fig. 1) was used on wounds without dressing. The presence of any petroleum based dressings prevents oxygen penetration into the wound. These are single use disposable devices

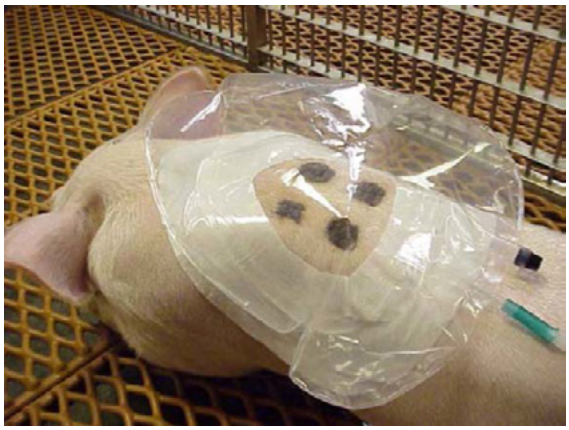

Fig. 1. Topical oxygen device affixed on pig dermal wounds. Photograph showing four wounds treated in a pig that was used to standardize the oxygen application approach used in the current study.

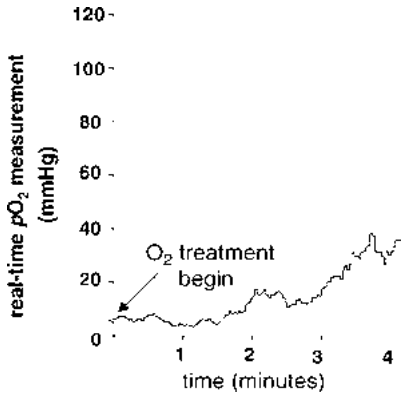

Fig. 2. Wound-bed pO_2 measurements in pigs treated or not with topical oxygen. The dermal wound model is described in this figure. pO_2 measurement was performed non-invasively using Oxy-Lite (Oxford-Optronix). An O_2 electrode was specially designed for our application (pO_2 assay at 2 mm depth) by the vendor. A real-time measurement of pO_2 in response to topical oxygen application is shown. The arrow indicates the time of initiation of topical O_2 treatment.

that come as sacral devices. They have an adhesive strip for fixation of the device to the skin. The device is connected to an oxygen gas cylinder. Initially, the bag is fully insufflated at high pressure. Subsequently, flow is initiated at 3–6 l/min. Each device has a release valve to prevent excessive pressure build-up within the bag. Although topical oxygen therapy for wounds has been used clinically in numerous wound care centers, the literature contains no direct report testing the effect of topical oxygen application on wound tissue pO_2. Exposure of open dermal wounds to topical oxygen treatment did not influence deep tissue pO_2 acutely. However, using a probe, specially designed to measure superficial pO_2 at 2 mm depth, topical application of pure oxygen slowly elevated wound bed pO_2 (Fig. 2). Note that this pO_2 reading reflects superficial wound tissue oxygen tension at the center of the wound bed and is not comparable to the routine clinical transcutaneous oxygen measurement (TCOM).

Repeated treatment of the excisional dermal wounds in pigs clearly accelerated wound closure in the early post-wound phase. This early advantage was maintained during the subsequent phase resulting in a significant acceleration of wound closure (Fig. 3). To test the quality of the regenerated tissue, we performed Masson-Trichrome and Hematoxylin-Eosin (H&E) staining of the wound-edge tissue on day 22 post-wounding. A broad region of hyperproliferative epithelium is a hallmark of the dermal wound edge. As the healing matures, this region narrows until it is reduced to a very thin margin typically observed in the intact skin. Both H&E as well as trichrome staining consistently revealed that the wounds treated with topical oxygen were in a more advanced stage of healing. The section of the regenerated tissue from wound treated with oxygen had a narrower hyperproliferative epithelium region compared to that in the tissue from the wound of the room air exposed wounds (Fig. 4). The expression of distinct keratin pairs during epidermal differentiation is assumed to fulfill specific and essential cytoskeletal functions. Keratin 14 plays a key role in epidermal remodeling. The intact skin stains positive for a thin epithelial band of keratin 14. Incomplete healing is associated with a broader distribution of keratin 14 in the healing skin along the hyperproliferative epithelium. As the healing matures and the hyperproliferative epithelium region narrows, the keratin 14 positive band becomes narrower and is pressed against the epidermis. Our results from keratin-14 staining of the regenerated tissue confirmed that indeed the wounds treated with oxygen presented histological signs of a higher maturity in healing compared to the tissues studied from the edge of the room-air treated wounds (Fig. 5). Immunohistochemical studies revealed a stronger presence of VEGF in the tissue from oxygen treated wounds compared to the

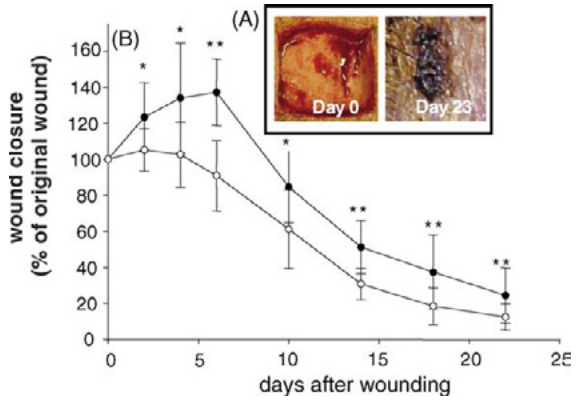

Fig. 3. Full-thickness dermal wound closure in response to topical oxygen administration in pigs. Ten (two clusters of five; on the back) secondary-intention full-thickness excisional dermal wounds (1 × 1 in.) were inflicted. Digital images of a typical wound on days 0 and 23 after wounding are shown in the inset. Five of ten wounds in each pig were treated with pure oxygen (open circles) for 3 h using a topical oxygen treatment device at a flow rate of 3–6 l/min. This treatment was performed every day for the first 7 days (day 0–6) from the day of wounding. Five of the control wounds (solid circles) were exposed to room air for the similar period. After treatment, wounds were dressed with moist Tegaderm dressing firmly held in place by Elasticon tape wrapped around the body. Digital imaging of wound was performed on days of oxygen treatment and every 4 days (during changes of wound dressing) following the treatment phase. One of the five wound in the treatment and placebo group was used for collection of biopsy. Images were analyzed using WoundMatrix® software. Mean ± S.D. $^{*}p < 0.05$; $^{**}p < 0.005$. Significantly smaller compared to corresponding control wounds.

room air exposed controls (Fig. 6A). Smooth muscle actin represents an integral component of blood vessel wall. Dual fluorescence staining of the tissue sections for smooth muscle actin and cell nuclei (DAPI, red) showed that the edge of oxygen treated wounds had a higher density of blood vessels than that in the edge of the room air exposed control wounds (Fig. 6B). While occurrence of blood vessels is indicative of angiogenesis, it is not a functional measure of vascularization. Tissue oxygen tension was chosen as a functional marker of the extent of vascularization. A well vascularized tissue is expected to have higher oxygen tension compared to a tissue with limited vasculature. Wound site pO_2 was assessed in both oxygen treated and room air exposed wounds. The analysis was carried out under resting conditions when oxygen treatment was not in progress. Results from such analyses showed that the oxygen treated wounds have better vascularization than room air treated control wounds (Fig. 6C).

4. Discussion

Wound healing is a multi-factorial process. Impairment of this process can be caused by the inadequacy of or lack of synchrony between multiple critical factors. It is widely acknowledged that limited oxygenation of the wound site is one key factor that results in wound chronicity. Angiogenesis is a rate-limiting factor in wound healing [27]. Oxygen and its reactive derivative hydrogen peroxide are known to induce angiogenic responses such as the induction of VEGF expression [24,25,28]. While hypoxia can initiate neovascularization by inducing angiogenic factor expression, it cannot sustain it. Acutely, hypoxia facilitates the angiogenic process [29] while chronic hypoxia impairs wound angiogenesis [30]. Sustained hypoxia causes death and dysfunction of tissue. Supplemental O_2 administration accelerates vessel growth [31]. VEGF is a major long-term angiogenic stimulus at the wound site. O_2 treatment induces VEGF mRNA levels in endothelial cells and macrophages [32–34] and increases VEGF protein expression in wounds in vivo [35]. Recently, it has been observed that O_2 may trigger the differentiation of fibroblasts to myofibroblasts [26], cells responsible for wound contraction.

Collagen deposition is a fundamental step in wound healing that provides the matrix for angiogenesis and tissue remodeling. There are several post-translational steps in collagen synthesis that are directly O_2 dependent. The enzymes prolyl hydroxylase, lysyl hydroxylase and lysyl oxidase all require molecular O_2 as a cofactor. Prolyl hydroxylase is required to convert proline residues to hydroxyproline, which allows the procollagen peptide chains to assume their triple helix configuration. Without this triple helix configuration, the synthesized procollagen chains accumulate in the rough endoplasmic reticulum and are eventually excreted as non-functional gelatinous protein [36]. Once the procollagen has assumed the triple helix conformation and has been excreted, the individual collagen fibers are arranged into linear fibrils via cross-linking of lysyl hydroxyalse and finally cross-linking between large fibrils is performed by lysyl oxidase. These extracellular cross-linkages are ultimately

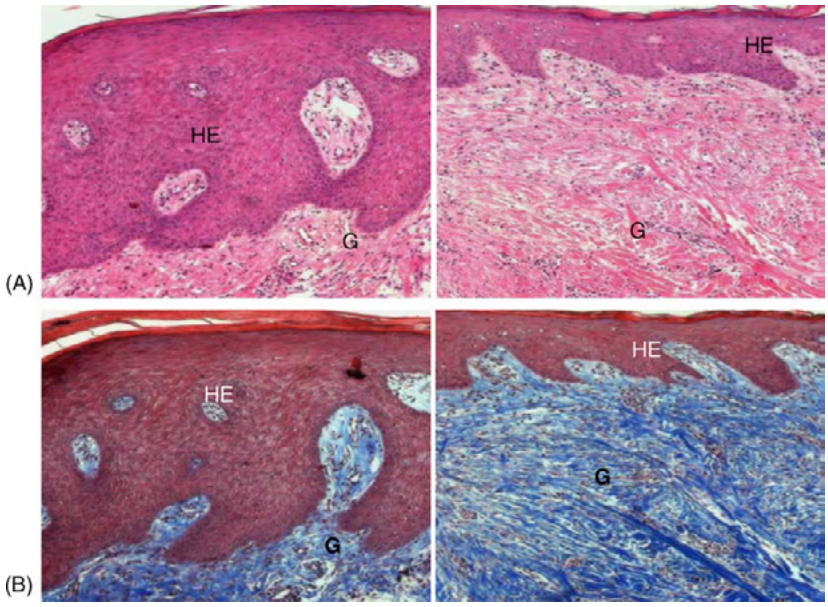

Fig. 4. Pig dermal wound histology in response to oxygen treatment. The dermal wound model is described above in Fig. 2. Three millimetres punch biopsies of the regenerated tissue were taken on day 22 from control and treated wounds. Formalin fixed paraffin sections were stained using (A) H&E or (B) Mason Trichrome. Note the architectural differences in the epidermis between the control and treated wounds, supporting advanced remodeling and healing in the treated as compared to the control group. HE, hyperproliferative epidermis; G, granulation tissue.

responsible for the tensile strength achieved in healed wounds. Of the O_2 dependent enzymatic processes, the rate of collagen synthesis is reflected by the rate at which prolyl hydroxylation occurs [36]. The amount of O_2 at which collagen synthesis is half-maximal (K_m using Micahelis-Menton equation) has been determined to occur at a pO_2 of 20–25 mmHg [37,38], with V_{max} occurring at levels approaching 250 mmHg. This represents levels of O_2 availability that exceeds the pO_2 normally present in wounds and suggests that adequate wound tissue oxygenation is crucial to support collagen synthesis. Indeed, increasing wound oxygenation results in increased collagen deposition and tensile strength [39–41].

Wound tissue oxygenation is an extremely sensitive indicator for the risk of infection in surgical patients [21,42]. The ability of supplemental O_2 to reduce infection is mediated by reactive oxygen species (ROS) such as H_2O_2 generated by NADPH oxidases in wound neutrophils and macrophages. The concentration of O_2 necessary to achieve half maximal ROS production (the K_m) is in the range of 45–80 mmHg, with maximal ROS production seen at pO_2 at >300 mmHg [30]. Thus, just as with the enzymes regulating collagen synthesis, the maximal effects of this biologic process can only be achieved through the administration of supplemental O_2 to attain wound pO_2 levels

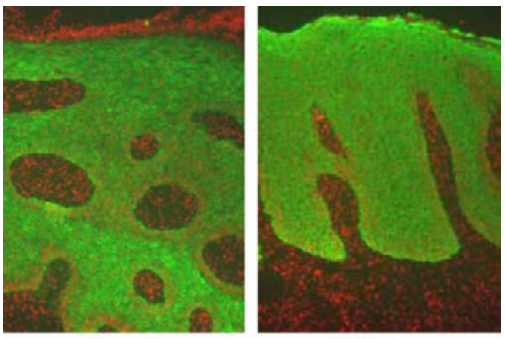

Fig. 5. Effect of oxygen treatment on epidermal remodeling during the healing process. The dermal wound model is described above in Fig. 2. Three millimetres punch biopsies were taken on day 15 from control and treated wounds. Formalin fixed paraffin sections were stained using antibody against keratin-14 (green) to stain for epidermis. Nuclei were stained with DAPI (red). Note more defined epidermis in treated side compared to the control.

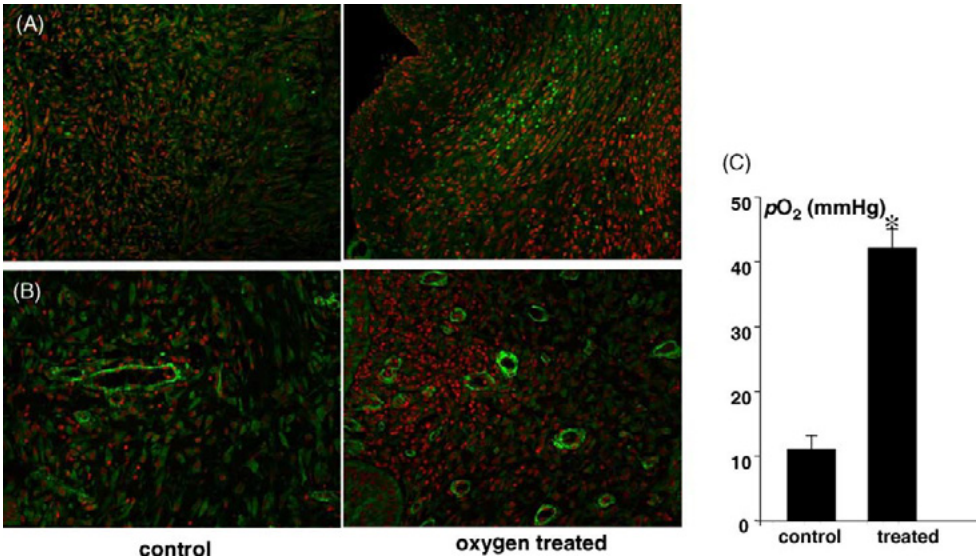

Fig. 6. Angiogenic response at the wound site following topical oxygen treatment of full-thickness dermal wounds. The dermal wound model is described above in Fig. 2. Three mm punch biopsy from wound margins were harvested. Formalin-fixed paraffin sections were stained using antibody against (A) vascular endothelial growth factor (VEGF, green, day 7 post wounding) or (B) α-smooth muscle actin (SMA, green, day 16 post wounding). Counterstaining of nuclei was performed using DAPI (red). Note that compared to the control side more VEGF and SMA stain in the treated side; (C) wound site pO_2 levels were measured under resting conditions on day 22. Mean ± S.D. $^{*}p < 0.05$. Baseline skin $pO_2 = 40–50$ mm Hg.

beyond those encountered when breathing room air. In fact, approximately 98% of the O_2 consumed by wound neutrophils and macrophages is utilized for respiratory burst [30]. At the wound-site, ROS are generated from oxygen by almost all wound-related cells. Recently, first evidence indicating that ROS may contribute to several facets of wound healing including angiogenesis has been reported [18,24,43]. Of importance, numerous wound healing related growth factors including PDGFβ (Regranex gel, Johnson & Johnson, Indianapolis, IN) rely on ROS for the execution of its biological function [44]. Oxidation plays a central role in promoting TGFβ function [26]. Indeed, strategies to raise wound pO_2 show a synergistic effect to benefit wound healing in conjunction with both TGFβ as well as PDGF therapy of wounds [45]. Fig. 7 presents a schematic illustration of the oxygen and ROS-sensitive pathways that are relevant to the current study.

From a diagnostic standpoint, many surgeons already use measurements of wound oxygenation to guide their treatment planning when they obtain TCOM with non-invasive vascular studies. TCOM measurements provide reliable prognostic information regarding the ability of wounds to heal and this has been used to determine amputation levels [17,19,46]. It is important to note though that TCOM does not reflect wound-site pO_2 like we have measured by placing a probe directly at the center of the wound. Standard TCOM measurements are conducted under conditions where the skin is warmed to 42 °C. This warmth factor contributes to overestimation of pO_2 especially because typically O_2 therapy to the wound is not accompanied with warming of the wound site [2]. There is a fundamental difference between the intact skin in the perimeter of the wound compared to the wound core. While the former is well vascularized, wound cores are typically characterized by disrupted vasculature and therefore suffer from poor blood perfusion. pO_2 measurement performed in this study and TCOM has another significant contrasting feature. TCOM is based on the Clark electrode technology [47]. This technology is particularly not best suited under hypoxic conditions because it consumes oxygen while measuring it. This may lead to artifacts especially under conditions where oxygen availability is limited [17]. In contrast,

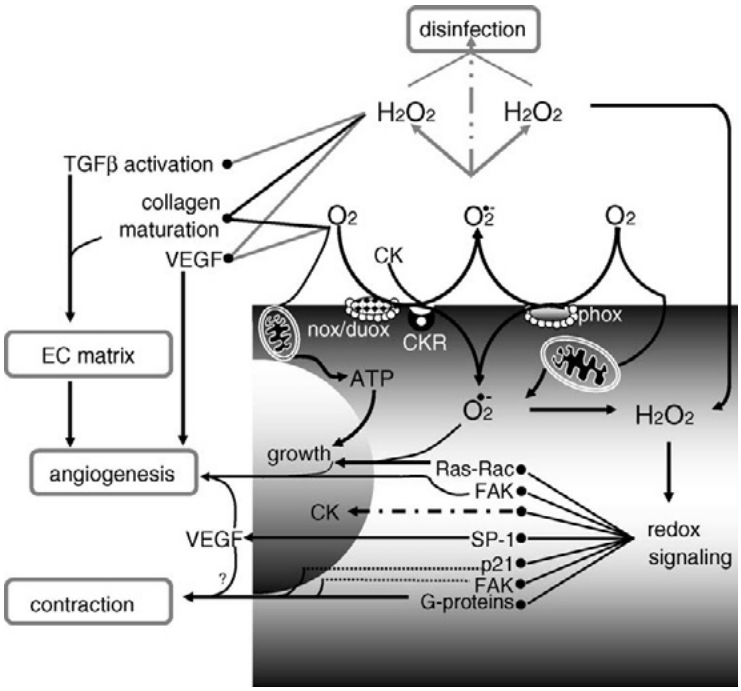

Fig. 7. Schematic illustration of select possible pathways by which oxygen and its reactive derivatives may influence wound healing related processes. The specific processes have been recently reviewed [43]. Excess generation of ROS, such as in cases where the inflammatory phase is not resolved in a timely manner, may cause oxidative damage and impair healing. CK, cytokine; CKR, cytokine receptor; EC, extracellular; FAK, focal adhesion kinase; phox, phagocytic NADPH oxidases; nox/duox, non-phagocytic oxidases.

the oxymetry system we employed is based on fiber-optics pO_2 probes which provide a continuous measure of O_2 partial pressure coupled with fast (<5 s) response times for real-time monitoring of temporal O_2 changes [48]. Fluorescence lifetime is longest at low pO_2, making these probes most sensitive in the physiological range 0–60 mmHg. Also, because the measurement is based on fluorescence lifetime rather than fluorescent intensity it is much less prone to artifacts (e.g. because of variation in the intensity of the light source, ambient lighting, or photo-bleaching). Compensation for the effects of temperature is required since fluorescent lifetimes are affected by changes in temperature. Temperature is measured by a fully integrated thermocouple, allowing simultaneous monitoring of tissue pO_2 and temperature as well as automatic temperature correction.

Results of this pre-clinical study present first evidence indicating that topical applied pure oxygen is capable of oxygenating the superficial wound tissue but not deep tissue. Because regeneration of new tissue is expected at the wound surface, it is reasonable to conclude that topical application of oxygen to open wounds had some favorable impact on the overall healing process. These findings suggest that treatment of open wounds with topical oxygen may provide beneficial results provided supply of oxygen to the superficial wound tissue is the key limiting factor. This hypothesis is consistent with previously reported clinical observation that topical oxygen treatment seems to be effective in many but not all cases [19]. If proven to be effective, topical O_2 therapy has the added advantage of caring for much larger potential patient population especially under conditions of public disaster and in a field-setting where HBOT may not be applicable. In addition, topical oxygen based therapeutics has the potential to bypass HBOT related risk of systemic toxicity [20,49]. Further studies testing the potential of topical oxygen in pre-clinical and clinical settings are warranted.

Acknowledgment

Supported by P50 GM27345 and RO1GM 69589 to C.K.S.

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PERSPECTIVE ARTICLE

Wound healing essentials: Let there be oxygen

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Manuscript received: February 5, 2008
Accepted in final form: October 12, 2008

DOI:10.1111/j.1524-475X.2008.00436.x

ABSTRACT

The state of wound oxygenation is a key determinant of healing outcomes. From a diagnostic standpoint, measurements of wound oxygenation are commonly used to guide treatment planning such as amputation decision. In preventive applications, optimizing wound perfusion and providing supplemental O_2 in the perioperative period reduces the incidence of postoperative infections. Correction of wound pO_2 may, by itself, trigger some healing responses. Importantly, approaches to correct wound pO_2 favorably influence outcomes of other therapies such as responsiveness to growth factors and acceptance of grafts. Chronic ischemic wounds are essentially hypoxic. Primarily based on the tumor literature, hypoxia is generally viewed as being angiogenic. This is true with the condition that hypoxia be acute and mild to modest in magnitude. Extreme near-anoxic hypoxia, as commonly noted in problem wounds, is not compatible with tissue repair. Adequate wound tissue oxygenation is required but may not be sufficient to favorably influence healing outcomes. Success in wound care may be improved by a personalized health care approach. The key lies in our ability to specifically identify the key limitations of a given wound and in developing a multifaceted strategy to specifically address those limitations. In considering approaches to oxygenate the wound tissue it is important to recognize that both too little as well as too much may impede the healing process. Oxygen dosing based on the specific need of a wound therefore seems prudent. Therapeutic approaches targeting the oxygen sensing and redox signaling pathways are promising.

The clinical application of O_2 to wound healing occurs at many levels: diagnostic, preventive, and therapeutic. From a diagnostic standpoint, measurements of wound oxygenation (transcutaneous O_2 measurements or TCOM) are commonly used to guide treatment planning such as amputation decision.[1–6] In preventive applications, optimizing wound perfusion and providing supplemental O_2 in the perioperative period reduces the incidence of postoperative infections.[7–9] Correction of wound pO_2 (partial pressure of oxygen in the wound tissue) may, by itself, trigger some healing responses.[10–18] More importantly, approaches to correct wound pO_2 favorably influence outcomes of other therapies such as responsiveness to growth factors and acceptance of grafts.[10,19,20] This leads to the concept of correction of wound hypoxia as adjunct to other therapeutic modalities.[14,21] Although the case for therapeutic approaches aimed at correcting wound tissue hypoxia is compelling, outcomes in the wound clinics have been inconsistent. The objective of this review article is to concisely address some of the fundamental and emergent concepts in tissue O_2 sensing and response with the goal to illuminate salient complexities and perform critical analysis of what should help improve clinical outcomes in response to O_2-based therapeutics.

WOUND ISCHEMIA AND HYPOXIA

Vascular complications commonly associated with problematic wounds are primarily responsible for wound ischemia. Limitations in the ability of the vasculature to deliver O_2-rich blood to the wound tissue leads to, among other consequences, hypoxia. Hypoxia represents a reduction in oxygen delivery below tissue demand, whereas ischemia is a lack of perfusion, characterized not only by hypoxia but also by insufficient nutrient supply. Hypoxia, by definition, is a relative term. It is defined by a lower tissue partial pressure of oxygen (pO_2) compared with the pO_2 to which the specific tissue element in question is adjusted to under healthy conditions in vivo. Depending on the magnitude, cells confronting hypoxic challenge either induce an adaptive response that includes increasing the rates of glycolysis and conserve energy or suffocate to death.[22] Generally, acute mild to moderate hypoxia supports adaptation and survival. In contrast, chronic extreme hypoxia leads to tissue loss. While the tumor tissue is metabolically designed to thrive under conditions of hypoxia,[23] hypoxia of the wound primarily caused by vascular limitations is intensified by coincident conditions (e.g., infection, pain, anxiety, and hyperthermia) and leads to poor healing outcomes.[24,25]

Three major factors may contribute to wound tissue hypoxia: (i) peripheral vascular diseases (PVDs) garroting O_2 supply, (ii) increased O_2 demand of the healing tissue, and (iii) generation of reactive oxygen species (ROS) by way of respiratory burst and for redox signaling (Figure 1). Other related factors such as arterial hypoxia (e.g., pulmonary fibrosis or pneumonia, sympathetic response to pain, hypothermia, anemia caused by major blood loss, cyanotic

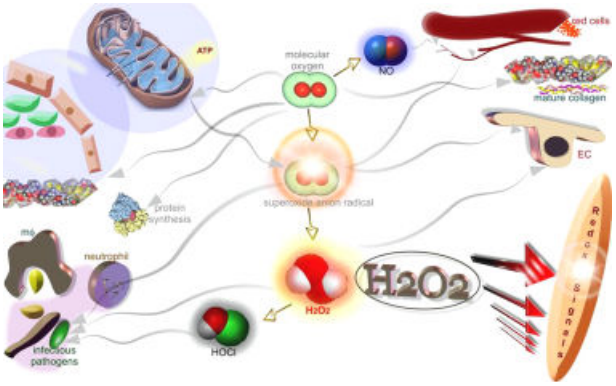

Figure 1. Significance of molecular oxygen and its derivatives in wound healing. In its molecular form, oxygen is required for oxidative metabolism-derived energy synthesis, protein synthesis, and the maturation (hydroxylation) of extracellular matrices such as collagen. Molecular oxygen is also required for NO synthesis, which in turn plays a key role in the regulation of vascular tone as well as in angiogenesis. In a wound setting, large amounts of molecular oxygen are partially reduced to form reactive oxygen species (ROS). ROS includes oxygen free radicals such as superoxide anion as well its nonradical derivative hydrogen peroxide (H_2O_2). Superoxide anion radical is the one electron reduction product of oxygen. NADPH oxidases represent one major source of superoxide anion radicals at the wound site. NADPH oxidases in phagocytic cells help fight infection. Superoxide anion also drives endothelial cell signaling such as required during angiogenesis. In biological tissues, superoxide anion radical rapidly dismutates to hydrogen peroxide—either spontaneously or facilitated by enzymes called superoxide dismutases. Endogenous hydrogen peroxide drives redox signaling, a molecular network of signal propagation that supports key aspects of wound healing such as cell migration, proliferation, and angiogenesis. Neutrophil-derived hydrogen peroxide may be utilized by myeloperoxidase to mediate peroxidation of chloride ions resulting in the formation of hypochlorous acid (HOCl), a potent disinfectant.

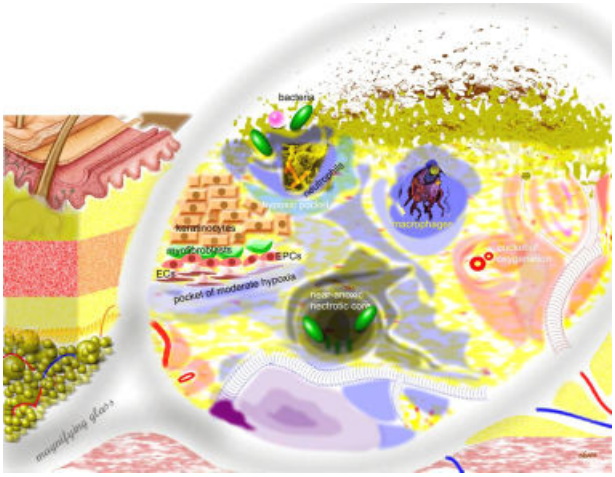

Figure 2. Heterogeneous distribution of oxygen in the wound tissue: hypothetical pockets of graded levels of hypoxia. Structures outside the illustrated magnifying glass represent the macro tissue structures. Objects under the glass represent a higher resolution. Shade of black (anoxia) or blue represents graded hypoxia. Shade of red or pink represents oxygenated tissue. Tissue around each blood vessel is dark pink in shade representing regions that are well oxygenated (oxygen-rich pockets). Bacteria and bacterial infection are presented by shades of green on the surface of the open wound.

heart disease, high altitude) may contribute to wound hypoxia as well. Depending on factors such as these, it is important to recognize that wound hypoxia may range anywhere from near-anoxia to mild–modest hypoxia.[26,27] In this context, it is also important to appreciate that point measurements[28] performed in the wound tissue may not provide a complete picture of the wound tissue biology because it is likely that the magnitude of wound hypoxia is not uniformly distributed throughout the affected tissue especially in large wounds. This is most likely the case in chronic wounds presented clinically as opposed to experimental wounds, which are more controlled and homogenous in nature. In any single problem wound presented in the clinic, it is likely that there are pockets of near-anoxic as well as that of different grades of hypoxia (Figure 2). As the weakest link in the chain, tissue at the near-anoxic pockets will be vulnerable to necrosis, which in turn may propagate secondary tissue damage and infection. Pockets of extreme hypoxia may be flooded with hypoxia-inducible angiogenic factors but would fail to functionally vascularize because of insufficient O_2 that is necessary to fuel the repair process. Indeed, uncontrolled expression of vascular endothelial growth factor (VEGF) and its receptors leads to insufficient skin angiogenesis.[29] Whether cells in the pockets of extreme hypoxia are O_2-responsive is another concern. Even if such cells may have passed the point of no return in the survival curve, correction of tissue oxygenation is likely to help clean up the dead or dying tissue[30,31] and replace the void with proliferating neighboring cells. Pockets of moderate or mild hypoxia are likely to be the point of origin of successful angiogenic response as long as other barriers such as infection and epigenetic alterations are kept to a minimum.

WOUND HYPOXIA: THE IMBALANCE BETWEEN LIMITED SUPPLY AND HIGH DEMAND

Limited supply: PVDs

PVD can affect the arteries, the veins, as well as the lymph vessels. The most common and important type of PVD is peripheral arterial disease (PAD), which affects about 8 million Americans. The ankle brachial pressure index represents a simple noninvasive method to detect arterial insufficiency within a limb. Arterial diseases, especially those associated with diabetes, represent a major complicating factor in wound healing. PAD is the only identifiable etiology in approximately 10% of leg ulcers.[32] In an ischemic limb, peripheral tissues are deprived of blood supply as PAD progresses causing tissue loss, ulcers, and gangrene.

Venous insufficiency, on the other hand, is the root cause of most leg ulcers.[33] Chronic venous insufficiency,

characterized by the retrograde flow of blood in the lower extremity, is associated with changes in the venous wall and valves generally caused by inflammatory disorders induced by venous hypertension and associated fluid shear stress. Factors causing arterial hypoxemia may also limit O_2 supply to the wound tissue. Compromised pulmonary health,[34] loss of hepatic function,[35,36] hemodialysis,[37] anemia,[38,39] altitude hypoxemia,[40] nitroglycerin therapy,[41] nasal packing,[42] critical illness,[43] pain,[44] and hypothermia[45,46] are some examples of conditions associated with arterial hypoxemia. Vasoconstricting drugs may contribute to tissue hypoxia as well.[47]

High demand: increased demand of the healing tissue

Mitochondrial respiration is responsible for more than 90% of O_2 consumption in humans. Cells utilize O_2 as the final electron acceptor in the aerobic metabolism of glucose to generate ATP, which fuels most active cellular processes such as during wound healing.[48] Increased energy demand of the healing tissue leads to a hypermetabolic state wherein additional energy is generated from oxidative metabolism increasing the O_2 demand of the healing tissue.[49–52] ATP thus generated powers tissue repair. At the injury site, extracellular ATP may be contributed by platelets and other disintegrating cells. Extracellular ATP liberated during hypoxia or inflammation can either signal directly to purinergic receptors or, after phosphohydrolytic metabolism, can activate surface adenosine receptors. Purinergic signaling may influence numerous aspects of wound biology including immune response, inflammation, vascular, as well as epithelial biology. ATP may be immunostimulatory or vice versa depending on extracellular concentrations as well as on expression patterns of purinergic receptors and ecto-enzymes.[53] Extracellular ATP induces receptor activation in epithelial cells. ATP, released upon epithelial injury, acts as an early signal to trigger cell responses including an increase in heparin-binding epidermal growth factor (EGF)-like growth factor shedding, subsequent transactivation of the EGF receptor and its downstream signaling, resulting in wound healing.[54] ATP released from the injured epithelial cells is now known to also turn on NADPH oxidases,[55] the activity of which is critically required to produce the redox signals required for wound healing.[19,56,57] Human endothelial cells are rich in purinergic receptors and therefore responsive to extracellular ATP as well.[58] ATP induces endothelium-dependent vasodilation.[59] Both ATP as well as adenosine regulate smooth muscle and endothelial cell proliferation.[60] Recognizing that hypoxia limits ATP synthesis in the ischemic wound tissue, therapeutic ATP delivery systems have been studied for their effect on wound healing.[61] While these approaches may compensate for the deficiency of ATP per se in the ischemic wound tissue, they will fail to address the other essential functions of O_2 and its derivatives in wound healing as discussed below.

Absolute requirements for O_2 arise in several points along the angiogenic sequence. For instance, all vessels require a net or sheath of extracellular matrix (ECM), mainly collagen and proteoglycans, to guide tube formation and resist the pressures of blood flow. Conditions for collagen deposition and polymerization can be created only if molecular O_2 is available to be incorporated into the structure of nascent collagen by prolyl and lysyl hydroxylases. Without the obligatory extracellular, hydroxylated collagen, new capillary tubes assemble poorly and remain fragile.[62–64] This has a convincing clinical correlate in scurvy, i.e., ascorbate deficiency. Scurvy may result from insufficient intake of ascorbate, which is required for correct collagen synthesis in humans. Ascorbate is required for the posttranslational hydroxylation of collagen that enables the matured collagen molecules to escape to the extracellular space and provide the necessary tensile strength.[65] In scurvy, the collagenous sheath cannot form because, under ascorbate-deficient conditions, collagen cannot be hydroxylated. Consequently, new vessels fail to mature. Older vessels weaken and break, and wounds fail to heal.[62] In this context, it is important to recognize that the collagen hydroxylation process requires molecular oxygen. Thus, even under ascorbate-sufficient conditions collagen may fail to mature if there is insufficient supply of oxygen to the tissue. Collagen deposition proceeds in direct proportion to pO_2 across the entire physiologic range, from 0 to hundreds of mmHg. The K_m for O_2 for this reaction is approximately 25 and the V_{max} is approximately 250 mmHg, suggesting that new vessels cannot even approach their greatest possible rate of growth unless the wound tissue pO_2 is high.[66] Angiogenesis is directly proportional to pO_2 in injured tissues.[63] Hypoxic wounds deposit collagen poorly and become infected easily, both of which are problems of considerable clinical significance.[67,68]

High demand: increased production of reactive species

Phagocytic NADPH oxidases

Sbarra and Karnovsky's 1959 discovery of the leukocyte oxidase[69] in phagocytes came into limelight in the late 1970s, when the pioneering works of Bernard Babior linked the explosive production of superoxide ions ($O_2^{\bullet -}$) by leukocyte oxidase to bacterial killing.[70] During phagocytosis of microbial intruders, professional phagocytes of our innate immune system increase their O_2 consumption through the inducible activity of NADPH oxidase (NOX) that generates $O_2^{\bullet -}$ and H_2O_2. These oxygen-derived metabolites give rise to yet other ROS that are potently antimicrobial but which may also cause damage by destroying surrounding tissue and cells. NADPH oxidase, catalyzing the deliberate production of ROS by cells, has been extensively investigated in phagocytes (neutrophilic and eosinophilic granulocytes, monocytes, and macrophages).[71] Exposure of these cells to any of a large number of stimuli activates a "respiratory burst," caused by an activation of the plasma membrane-bound NADPH oxidase ($NADPH + 2O_2 \rightarrow NADP^+ + 2O_2^{\bullet -} + H^+$). The $O_2^{\bullet -}$ then rapidly dismutates to H_2O_2. Approximately 98% of the O_2 consumed by wound neutrophils is utilized for respiratory burst.[24] NADPH oxidase supports macrophage survival[72] and enables dead cell cleansing by phagocytosis.[73] Appropriate infection management may therefore spare precious O_2 at the wound site, which would otherwise be utilized via respiratory burst.[74] Overt infection poses the risk of intensifying wound tissue hypoxia.

The NOX of "professional" phagocytic cells transfers electrons across the wall of the phagocytic vacuole, forming $O_2^{\bullet -}$ in the lumen. It is generally accepted that this

system promotes microbial killing through the generation of ROS and through the activity of myeloperoxidase.[75] In response to bacterial infection, the neutrophil NADPH oxidase assembles on phagolysosomes to catalyze the transfer of electrons from NADPH to O_2, forming $O_2^{\bullet -}$ and derivative ROS. The active oxidase is composed of a membrane-bound cytochrome (e.g., gp91phox and p22phox) together with three cytosolic phox proteins, p40phox, p47phox, and p67phox, and the small GTPase Rac2, and is regulated through a process involving protein kinase C, mitogen-activated protein kinase, and phosphatidylinositol 3-kinase.[76,77] In the resting cell, two of the subunits, p22phox and gp91phox, are located in the membrane, and the remaining components are present in the cytosol. The electron-carrying components of the oxidase are located in gp91phox.[78–81] The NADPH-binding site is generally regarded to be in gp91phox as well, but there is some evidence that it may be in p67phox. The catalytic subunit gp91phox, dormant in resting cells, becomes activated by assembly with cytosolic regulatory proteins. When the oxidase is activated, p47phox is phosphorylated at specific sites, and the cytosolic components together with Rac2 migrate to the membrane to assemble the active oxidase.[19] Mutations in p47phox are a cause of chronic granulomatous disease, an immune-deficient condition characterized with impaired healing response.[82,83] Rac2 mutation is another factor responsible for impaired human neutrophil NADPH oxidase function, low $O_2^{\bullet -}$ generation, and compromised wound healing.[84] The concentration of O_2 necessary to achieve half maximal ROS production (the K_m) is in the range of 45–80 mmHg, with maximal ROS production at pO_2 at > 300 mmHg.[24] Thus, the maximal effects of respiratory burst-dependent wound infection management can only be achieved through the administration of supplemental O_2 to attain wound pO_2 levels beyond those encountered when breathing room air.[85] This also explains why the state of wound tissue oxygenation is a sensitive indicator for the risk of infection in surgical patients.[8,9,86,87]

Oxygen free radicals and reactive derivatives: a paradigm shift and emergence of redox signaling

In the 1980s, oxygen free radicals drew much attention in biomedical research. Limitations in methodological approaches to sensitively detect and monitor the extremely short-living reactive species clouded a true appreciation of the significance of oxygen-derived free radicals and reactive species in health and disease. The paradigm that emerged was too simple to be meaningful in its complete sense. The primary identity of free radicals was that they were destructive to biological tissues, and that approaches to antagonize free radicals, i.e., antioxidants, are helpful.[88–96] Based on this crude preliminary concept, numerous clinical trials testing the efficacy of antioxidants were hastily started and the results were understandably disappointing.[97–101] Lack of consideration of a very important aspect of free radical biology that started to crystallize only in the late 1990s proved to be very expensive in many ways. Work during the mid-late 1990s led to the recognition that at very low levels, oxygen-derived free radicals and derivative species such as H_2O_2 may serve as signaling messengers.[102–104] The field of redox signaling was thus born[102,105–107] with a dedicated international peer-reviewed journal (http://www.liebertpub.com/ars). Today, the concept that reactive derivatives of O_2 may serve as signaling messengers has revolutionized cell biology[108–123] and has led to the concept of redox-based clinical therapeutics.[124–129]

Nonphagocytic NADPH oxidases

Given the traditional bad and ugly image of oxygen free radicals and its derivatives, few would have imagined that even nonphagocytic cells of the human body have a dedicated apparatus to generate ROS. In 1999, the cloning of Mox1 marked a major progress in categorically establishing the presence of distinct NADPH oxidases in nonphagocytic cells.[123] Mox1 or p65Mox was described as encoding a homolog of the catalytic subunit of the $O_2^{\bullet -}$-generating NADPH oxidase of phagocytes, gp91phox. Mox1 messenger RNA is expressed in colon, prostate, uterus, and vascular smooth muscle, but not in peripheral blood leukocytes. Later, Mox1 was renamed as NOX1 referring to NADPH oxidase.[130] Over the last years, six homologs of the cytochrome subunit of the phagocyte NADPH oxidase were found: NOX1, NOX3, NOX4, NOX5, DUOX1, and DUOX2. Together with the phagocyte NADPH oxidase itself (NOX2/gp91(phox)), the homologs are now referred to as the NOX family of NADPH oxidases. Activation mechanisms of these enzymes and tissue distribution of the different members of the family are markedly different. The physiological functions of NOX family enzymes include host defense, posttranslational processing of proteins, cellular signaling, regulation of gene expression, cell differentiation, and renewal of precursor cells.[131–135] NOX enzymes also contribute to a wide range of pathological processes. NOX deficiency may lead to immunosuppresion, lack of otoconogenesis, or hypothyroidism. Increased NOX activity also contributes to a large number or pathologies, in particular cardiovascular diseases and neurodegeneration.[136] Thus, optimal generation of $O_2^{\bullet -}$ is required to sustain healthy living.

Acute inflammation following injury is the site for abundant production of ROS by phagocytic NADPH oxidases. As inflammation resolves and phagocyte count at the wound site falls, several aspects of healing such as cell proliferation and migration are supported by redox signaling where low-level ROS produced by nonphagocytic oxidases serve as messenger molecules.[57] The critical significance of the NADPH oxidases in wound healing is rapidly unfolding. As discussed previously, NADPH oxidase-deficient mice and humans suffer from impaired healing. As an integral part of the healing response, wounding induces H_2O_2 production.[56] This response is also conserved in plants.[137] Wound fluid from healing tissues contains the highest concentration of H_2O_2 compared with all other bodily fluids.[56,138] Of note, selective decomposition of H_2O_2 at the wound site using catalase overexpression approaches impairs the healing process demonstrating the key significance of H_2O_2 in wound healing.[56] Importantly, catalase-dependent decomposition of H_2O_2 generates O_2 as end-product. Thus, molecular O_2 is not sufficient if NADPH oxidase-dependent O_2 consumption and redox signaling is impaired. How redox signals may contribute to

tissue repair has been recently reviewed elsewhere[57,139] and is beyond the scope of this article. In the context of this article, it is important to appreciate that redox signals are generated at the cost of tissue O_2. Thus, tissue hypoxia will limit redox signaling and disable the function of several growth factors (e.g., platelet-derived growth factor [PDGF], VEGF, keratinocyte growth factor, insulin-like growth factor, transforming growth factor-α) and numerous molecular mechanisms (e.g., leukocyte recruitment, cell motility, integrin function), which rely on redox signaling.[57,139,140]

Collagen deposition provides the matrix for angiogenesis and tissue remodeling. Maturation of collagen is O_2 dependent. Of the O_2-dependent enzymatic processes, the rate of collagen synthesis is reflected by the rate at which prolyl hydroxylation occurs.[141] Collagen synthesis is half-maximal (K_m using Micahelis–Menton equation) at a pO_2 of 20–25 mmHg,[66,142] with V_{max} at levels approaching 250 mmHg. This represents levels of O_2 availability that exceeds the pO_2 normally present in the wound tissue and suggests that adequate wound tissue oxygenation is crucial to support collagen synthesis and maturation. Indeed, increasing wound oxygenation results in increased collagen deposition and tensile strength.[143–145]

Nitric oxide (NO) synthases

NO is widely recognized as a major signaling messenger that drive numerous aspects of (patho)physiology.[146–149] O_2 consuming NO synthases (NOS) catalyze NO formation from the amino acid L-arginine. The reaction of NOS with O_2 is fast and takes place within several steps.[150] NOS are known to catalyze more than one reaction: the NO-producing reaction is considered to be the coupled reaction, and the uncoupled reactions are those that produce ROS, such as $O_2^{\bullet -}$ and H_2O_2.[151] The key significance of NO in wound healing has been reviewed elsewhere.[152,153] In the context of this article, it is important to note that O_2 is often the overlooked substrate in NO synthesis. To date, there has been little consideration of the role of O_2 tension in the regulation of NO production associated with wound healing. Tissue O_2 tension is known to significantly alter endogenous NO production in articular cartilage where the tissue pO_2 is comparable to that of ischemic wounds.[154] The preliminary observation that hyperbaric oxygen (HBO) therapy may significantly increase local wound NO levels is therefore understandable.[155] Once generated, the biological significance of NO also depends on the tissue oxygenation status.[156] As NO gas-based therapies are being considered for healing wounds clinically, it is important to recognize that NO can block mitochondrial function by interacting with the cytochrome *c* oxidase (complex IV) of the electron transport chain in a manner that is reversible and in competition with O_2. Concentrations of NO too low to inhibit respiration can trigger cellular defense response mechanisms. Inhibition of mitochondrial respiration by NO at low O_2 concentrations can cause so-called "metabolic hypoxia" and divert O_2 toward other oxygen-dependent systems. Metabolic hypoxia refers to a state wherein although O_2 is available the cell is unable to utilize it for respiration.[157] Such a diversion reactivates prolyl hydroxylases and thus accounts for the prevention by NO of the stabilization of the hypoxia-inducible factor (HIF). When NO inhibits mitochondrial respiration under hypoxia, it prevents mitochondria from depleting local oxygen, enabling the continued hydroxylation and degradation of HIF-1α, thus leading to a situation in which the cell may fail to register hypoxia. Furthermore, in a wound setting where $O_2^{\bullet -}$ production is highly active, NO is likely to generate peroxynitrite that can affect the action of key enzymes, such as mitochondrial complex I, by *S*-nitrosation.[157] NO-based wound therapeutics should be designed in light of these complexities.

The stability of HIF, and therefore its ability to drive HIF-dependent gene transcription, is differentially regulated by NO under conditions of normoxia and hypoxia. While NO stabilizes HIF under normoxia, the effect is exactly opposite under conditions of hypoxia.[158] Under conditions of normoxia, NO may attenuate the ubiquitination of HIF-1α and thus abrogate binding of von Hippel-Lindau (pVHL) to HIF-1α.[159] Ubiquitination of HIF would not take place if HIF is not hydroxylated by prolyl hydroxylase domain enzymes (PHDs). Indeed, NO inhibits PHD activity. Fe^{2+} coordination by NO seems to be the explanation for how NO inhibits PHDs. The stabilization of HIF under normoxia is also explained by the induction of HIF-1α synthesis by NO.[160] Although speculative, different redox-active products, derived from chemically distinct NO donors, use divergent transmission systems to stabilize/express HIF-1α.[160] Under conditions of hypoxia, NO and its derivatives inhibit hypoxia-induced HIF-1α accumulation.[158] In light of the observation that NO attenuates PHD activity under normoxia to stabilize HIF-1α, raises the question whether PHD activity is regained under conditions of hypoxia–NO coexistence. An affirmative answer to this question came from the observation that oxygen-dependent death domain of HIF-1α, which accounts for protein stability, is needed for NO and its derivatives to reverse hypoxic HIF-1α stabilization.[161] Several mechanistic hypotheses have been proposed to explain how NO impairs accumulation of HIF-1α under hypoxia.[158] The scenario gets even more complicated in a wound setting where both phagocytic as well as non-phagocytic NADPH oxidases generate copious amounts of superoxide anion radicals.[56,138] Furthermore, hypoxic tissues are known to generate more ROS. The HIF system has revealed an unexpectedly direct connection between molecular oxygen, superoxide, and NO in achieving or attenuating responses to hypoxia. The reaction between $O_2^{\bullet -}$ and NO represents a primary biochemical path in vivo.[162] Flux rates of NO and $O_2^{\bullet -}$, as well as the presence of antioxidant enzymes, can modulate HIF-1α stabilization.[158] Understanding the multiple signals, which have the potential to deliver a flexible and controlled response to hypoxia, will be critical to develop therapeutic maneuvers. Thus, a clear appreciation of the specific wound tissue redox environment[57] becomes critically important in the context of planning NO-based therapeutics.

THE NORMOXIC SETPOINT AND OXYGEN SENSING

Cellular O_2 homeostasis is tightly maintained within a narrow range ("normoxia") due to the risk of oxidative

damage from excess O_2 (hyperoxia), and of metabolic demise from insufficient O_2 (hypoxia). The vast majority of the current literature focuses on the sensing of hypoxia, and the work on hyperoxic sensing is limited. Both hypoxia and hyperoxia are relative terms. They refer to a state of oxygenation that departs from the normoxic setpoint, i.e., the pO_2 to which cells or tissues are adjusted to under basal conditions.[163] For any given cell or tissue, normoxic setpoint represents that state of oxygenation where the cell or tissue does not report hypoxia neither do they induce hyperoxia-induced cell signaling or manifest overt oxygen toxicity. It is likely that this setpoint would represent a range of pO_2, the span of which might depend on the tissue in question. Any change of O_2 ambience exceeding that span would result in the switching on of a hypoxic or hyperoxic response. In the finest of scales, such response would be detected in the molecular scale such as HIF stabilization or hypoxia response element (HRE) transactivation for hypoxia and say p21 induction for hyperoxia.[164,165] In a relatively coarser scale, oxygen-sensitive changes in cellular phenotype may be noted. Of note, different organs of the body have different normoxic setpoints. While the lung and arterial vasculature represent the high end, organs such as the liver have very low basal pO_2. pO_2 ranges from 90 to below 3 torr in mammalian organs under normoxic conditions with arterial pO_2 of about 100 torr or $\sim 14\%$ O_2.[166]

Hypoxia sensing

Hypoxia sensing and response is activated upon exposure to a state of oxygenation that is lower than the pO_2 to which the cells or tissue is adjusted to under basal conditions. This response cascade is centrally important in coping with the challenge of O_2 deficiency. Hypoxia response has been mostly studied in transformed and tumor cells. It is important to recognize that findings from such cells may not be directly applicable to nontransformed primary cells that are involved in wound healing.[167] Hypoxia is a hallmark of all ischemic diseases but is also noted under several physiological processes where exposure to a dynamic state of oxygenation is an integral component. During early pregnancy, trophoblast differentiation occurs in an environment of relative low O_2 tension, which is essential for normal embryonic and placental development.[168] O_2 supply to the human embryo in the first trimester is tightly controlled, suggesting that too much O_2 may interfere with development. Relative to maternal tissue pO_2, the embryo is normally in a state of partial hypoxia.[169,170] Thus, hypoxia sensing and response is not only implicated in ischemic disease conditions but is also required for development where a changing state of oxygenation seems to serve as a cue for successful development. Whether this is nature's approach to quality check each healthy birth for the ability of the new born to cope with ischemic diseases later on in their lives may be viewed as a matter of interesting speculation.

Hypoxia sensing and response mechanisms may be broadly classified into two general categories: HIF-dependent and HIF-independent. Extensive discussion of these pathways is beyond the scope of this article and the readers are referred to excellent review articles.[171–173]

HIF-dependent pathways

The basic helix–loop–helix (bHLH) proteins form a large superfamily of dimeric transcriptional regulators that are found in organisms from yeast to humans and function in critical developmental processes. One basis for the evolutionary classification of bHLH proteins is the presence or absence of additional domains, of which the most common are the PAS, orange, and leucine-zipper domains. PAS domains, located carboxy-terminal to the bHLH region, are 260–310 residues long and function as dimerization motifs. They allow binding with other PAS proteins, non-PAS proteins, and small molecules such as dioxin. The PAS domain is named after three proteins containing it: Drosophila Period (**P**er), the human aryl hydrocarbon receptor nuclear translocator (**A**rnt), and Drosophila Single-minded (**S**im). HIFs belong to the bHLH–PAS family of environmental sensors that bind to canonical DNA sequences called HREs in the promoters or enhancers of target genes.[174] HIF is able to direct transcription from either of two transactivation domains, each of which is regulated by distinct mechanisms. The O_2-dependent asparaginyl hydroxylase factor-inhibiting HIF-1α (FIH-1) is a key regulator of the HIF C-terminal transactivation domain, and provides a direct link between O_2 sensing and HIF-mediated transcription. Additionally, there are phosphorylation and nitrosylation events reported to modulate HIF transcriptional activity, as well as numerous transcriptional coactivators and other interacting proteins that together provide cell and tissue specificity of HIF target gene regulation.[175]

HIF-1 consists of a constitutively expressed subunit HIF-1β and an oxygen-regulated subunit HIF-1α (or its paralogs HIF-2α and HIF-3α). The transcriptional role of HIF is primarily dependent on the stabilization of HIF-1α or its paralogs under hypoxic conditions. Under O_2-replete conditions HIF-1α is very labile.[176] Molecular O_2 targets HIF for degradation by posttranslational hydroxylation at specific prolyl residues within the α subunits. Hydroxylation at two prolyl residues within the central degradation domain of HIF-1α increases the affinity for the pVHL E3 ligase complex by at least three orders of magnitude, thus directing HIF-α polypeptides for proteolytic destruction by the ubiquitin/proteasome pathway. Because the HIF hydroxylases have an absolute requirement for molecular O_2 this process is suppressed in hypoxia allowing HIF-α to escape destruction and activate transcription.

The O_2-sensitive PHDs and the asparagines hydroxylase (FIH) regulate the transcriptional activity of HIFs.[175] The unusual high K_m of PHDs for oxygen allows small changes in the oxygen supply to affect enzyme activity, which makes this system an ideal oxygen sensor. In hypoxia, FIH-1 hydroxylation of Asn803 within the C-terminal transactivation domain does not occur and HIF-1α fails to form a fully active transcriptional complex. Thus, HIF prolyl hydroxylation regulates proteolytic degradation of HIF whereas HIF asparaginyl hydroxylation modulates interaction with transcriptional coactivators. These hydroxylations are catalysed by a set of non-heme Fe(II)- and 2-oxoglutarate (2-OG)-dependent dioxygenases. During catalysis, the splitting of molecular O_2 is coupled to the hydroxylation of HIF and the oxidative decarboxylation of 2-OG to give succinate and CO_2. The von Hippel-

Lindau tumor suppressor gene product, pVHL, functions as the substrate recognition component of an E3-ubiquitin ligase, which targets the O_2-sensitive α-subunit of HIF for rapid proteasomal degradation under normoxic conditions and as such plays a central role in molecular O_2 sensing.

Stabilization of HIF under hypoxic conditions is followed by nuclear localization where HIF may bind to DNA sequences and other transcriptional regulators to influence gene expression (Table 1). The passage of transcription factors, e.g., HIF-1α into the nucleus through the nuclear pore complex is regulated by nuclear transport receptors. Therefore, nucleocytoplasmic shuttling can regulate transcriptional activity by facilitating the cellular traffic of transcription factors between both compartments.[177]

Shortly after the cloning of HIF-1α, a closely related protein, HIF-2α (also known as endothelial PAS protein, HIF-like factor, HIF-related factor, and member of the PAS superfamily 2), was identified and cloned.[178]

HIF-2α regulates erythropoietin production in adults.[179] HIF-1α functions as an upstream player in the p21-mediated growth arrest of keratinocytes.[180] Thus, HIF may antagonize certain aspects of skin repair. Negative pressure wound therapy, known to be effective in healing wounds clinically, is known to antagonize the stabilization of HIF-1α.[181] HIF-dependent pathways for survival and vascularization can function under conditions where hypoxia is moderate and not extreme. As long as there is a threshold level of oxygenation sufficient to sustain life, HIF-dependent survival responses may benefit wound healing.[182–184] Near-anoxic hypoxia, often noted in problem wounds,[26,27] is not compatible with life or tissue repair.

HIF-independent pathways

Conservation of ATP under conditions of limited O_2 supply is a HIF-independent survival response that is not compatible with the energy-demanding healing process.[49] For example, HIF-independent hypoxic inhibition of protein synthesis and cell growth is mediated by (i) hypoxia-induced cellular energy depletion; (ii) mTOR inhibition via the AMP-activated protein kinase (AMPK)/TSC2/Rheb pathway; (iii) eEF2 inhibition mediated by AMPK; and (iv) induction of endoplasmic reticulum (ER) stress that leads to eIF2α inhibition.[185] mTOR is a Ser/Thr kinase that integrates signals from growth factors and nutrients to increase ribosome biogenesis.[186] Upon hypoxic energy starvation, AMPK phosphorylates eEF2 kinase (eEF2K) on Ser398 and activates its kinase activity.[187] eEF2K then phosphorylates elongation factor eEF2 at Thr56, resulting in the inhibition of peptide elongation. mRNA translation is a critical component of cell growth and proliferation that is critically supported by eIF2α. Hypoxia causes ER stress, which in turn inhibits eIF2α.[185] Wound healing requires protein synthesis.[188–190] Hypoxia causes global down-regulation of protein synthesis. Hypoxia-induced translational attenuation may be linked to ER stress and the unfolded protein response.[191] The translational efficiency of individual genes is dynamic and changes with alterations in the cellular environment.[192] Whereas changes in transcription can take hours to achieve, translational regulation is rapid and reversible.[193] Preferential translation of select mRNA is another hallmark of response to hypoxia. Roughly 2.5% of total cellular transcripts are preferentially translated, despite arrest of global protein synthesis, in response to sustained extreme hypoxia.[194] Taken together, while all these hypoxia responses

Table 1. Hypoxia-inducible factor-1 (HIF-1) target genes

Erythropoiesis/iron metabolism	Cell survival/ proliferation	Angiogenesis	Vascular tone	Glucose metabolism	Matrix metabolism
EPO	IGF-2	VEGF	NOS2	HK1,2	MMPs
Tf	TGF-α	Leptin	HO1	LDHA	PAR/PAI
Tfr	ADM	TGF-β3	ET1	PKM	Coll PHD
Ceruloplasmin	BNip3	EG-VEGF	ADM	PFKL	
	NIX		α_{1b}	PGK1	
	NDRG2			PFKFB3	
				GAPDH	
				GLUT1,3	
				ENO1	
				CA-9	
				ALD-A,C	
				AK-3	

α_{1b}, α_{1b}–adrenergic receptor; ADM, adrenomedulin; AK, adenylate kinase; ALD, aldolase; BNip3, Bcl-2/adenovirus EIB 19kD-interacting protein 3; CA, carbonic anhydrase; Coll PHD, collagen prolylhydroxylases; EG-VEGF, endocrine gland-derived VEGF; ENO, enolase; EPO, erythropoietin; ET, endothelin; GAPDH, gylceraldehyde phosphate dehydrogenase; GLUT, glucose transporters; HK1,2, hexokinase 1,2; HO, heme oxygenase; IGF, insulin-like growth factor; LDH-A, lactate dehydrogenase-A; MMP, matrix metalloproteinases; NDRG, N-Myc downstream-regulated genes; NIX, Nip 3-like protein X; NOS, nitric oxide synthase; PAR/PAI, plasminogen activator receptors and inhibitors; PGK1, phosphoglycerate kinase 1; PFKL, phosphofructokinase L; PKM, pyruvate kinase M; TGF, transforming growth factor; TF, transferrin; Tfr, Tf receptor.

Source: http://rulai.cshl.edu/TRED/GRN/HIF.htm

represent important HIF-independent mechanisms of energy conservation that promote survival under low O_2 conditions, they are not compatible with the formation of new tissue as required during wound healing.

Intermittent hypoxia (IH)

O_2 sensing is no longer a unique property limited to chemoreceptors but is a common property of tissues.[195] The classic concept of IH has been markedly revised in light of our current understanding of O_2 sensing. IH, or periodic exposure to hypoxia interrupted by return to normoxia or less hypoxic conditions, occurs in many circumstances. Chronic intermittent hypoxia (CIH) is a common life-threatening condition that occurs in many different diseases, including sleep-disordered breathing manifested as recurrent apneas. Excessive ROS have been identified as one of the causative factors in a variety of morbidities.[196] In experimental models, CIH activates ROS-dependent responses that include (a) altered carotid body function, the primary chemoreceptor for sensing changes in arterial blood O_2; (b) elevated blood pressure; (c) enhanced release of transmitters and neurotrophic factors; (d) altered sleep and cognitive behaviors; and (e) activation of second-messenger pathways and transcriptional factors. Considerable evidence indicates elevated ROS levels in patients experiencing CIH as a consequence of recurrent apneas.[196] Recently, we evaluated the prevalence of obstructive sleep apnea (OSA) in the patient population of the OSU Wound Center. Between August 15 and September 30, 2007, 105 consecutive unscreened patients of the wound center completed a sleep screening questionnaire. In this representative sample of patients of the wound center, 51% either were diagnosed with, or were at very high risk for OSA. Forty-three percent of patients with chronic nonhealing wound were deemed at high risk for OSA.[197] Whether IH associated with OSA in chronic wound patients complicates wound healing warrants further investigation. Results of our survey may be explained by the association that many with chronic wounds are overweight due to metabolic complications (e.g., PAD and type II diabetes), and sleep apnea is more prevalent in overweight individuals. Merit of the hypothesis that sleep disorder may complicate wound healing is supported by the extensive literature identifying OSA as a causative factor underlying vascular disorders.[198,199]

Hyperoxia sensing

O_2 got its name from ''Principe Oxygene,'' which means the acidifying principle. ''Oxy'' is from Greek, and means sharp or acid; ''gen'' is also from Greek, and means the origin of. Taken together, oxygen means ''the origin of acid.'' Joseph Priestly's (1774) ''dephlogisticated air''[200] and Carl Scheele's (1771) ''fire air'' were soon characterized by Antoine Lavoisier as pure respirable air.[201] Within decades of the first realization that oxygen is the element of life, Brizé-Fradin[202] noted in 1808 that ''vital air'' or pure oxygen would soon wear life out instead of maintaining it. That oxygen may be harmful to human health was first postulated in the late 19th century with Paul Bert's work (1878) on oxygen sickness. Paul Bert's work is regarded as one of the cornerstones of HBO medicine.[203] He concluded that to avoid harmful effects, oxygen should not be inhaled at a concentration above 60% at 1 ATA. Bert's observation was extended through Michaeli's theoretical considerations, Gerschman's experimental verification, and finally caught the interests of biomedical scientists when in 1969 McCord and Fridovich demonstrated that a metalloenzyme produced H_2O_2 by combining $O_2^{\bullet -}$ with hydrogen.[204,205] Today, H_2O_2 is widely known to function as a cellular messenger.[108–123] Hyperoxia-inducible molecular biomarkers have been characterized[164,165] enabling us to detect hyperoxic insult long before overt signs of oxygen toxicity and adverse clinical symptoms are manifested.[206]

Although marginal hyperoxic challenge may induce favorable responses,[207] a state of tissue oxygenation that far exceeds the normoxic setpoint of a given tissue is a clear risk factor that deserves appropriate attention.[208] In a wound with pockets of hypoxia ranging in magnitude from extreme to marginal (Figure 2), the goal should be to reestablish normoxia in the worst affected hypoxic pockets without exposing other parts of the wound tissue to such high levels of pO_2 that would antagonize healing by hyperoxia-induced growth arrest or simply overt oxygen toxicity. One needs to be cautious about too much of a good thing.[209] Endothelial progenitor cells (EPCs) are essential in vasculogenesis and wound healing, but their circulating and wound level numbers are decreased in diabetes. Hyperoxia reverses the diabetic defect in EPC mobilization.[210] Moderate hyperoxia increases the appearance of new blood vessels in wounds.[11] In addition to inducing VEGF gene expression, moderate hyperoxia enhances the expression of $VEGF_{121/165}$ proteins and facilitates the release of $VEGF_{165}$ from cell-associated stores.[211] Among the factors that may oppose wound healing, extreme hyperoxia causes growth arrest[212–215] and cell death by a mitochondria-dependent apoptosis pathway.[171,216,217] In addition, extreme hyperoxia does pose the threat of oxidative stress.[218,219]

Tuning the normoxic setpoint

When cells grown under standard culture conditions of 20% O_2 are moved to 5% O_2 ambience, hypoxia is reported by way of HIF-response elements. When the same cells are maintained at 5% O_2 over long periods of time, the O_2-sensitive molecular machinery undergoes adjustment such that the same cells no longer report hypoxia. Interestingly, if these cells are maintained under mild hyperoxic conditions, e.g., 30% O_2, and then brought down to 20% O_2 culture conditions they report hypoxia.[163] These simple observations establish two important points: (i) that it is not the actual pO_2 but the ΔpO_2 that seems to matter; and (ii) that the normoxic setpoint in a cell can be reset by the adjustment of O_2-sensing machinery that is capable of responding to changes in the O_2 ambience. In this simplified example, the machinery is represented by the PHD family of proteins, the expression of which is up-regulated under conditions of hypoxia and down-regulated under conditions of hyperoxia. This is noted not only in vitro but also in vivo. Here, although the example is limited to PHDs to keep the discussion simple, it is important to recognize that there are numerous other O_2-sensitive functions in a cell that would contribute to its overall response to any pO_2 outside the normoxic setpoint. Thus, the normoxic setpoint in a

biological cell is tunable. For example, under conditions of no change in ambient O_2 condition, a cell may be made to report hypoxia, as measured by HIF transactivation, simply by knock-down of the PHDs.[163] In response to down-regulated PHD1, cells not only report HRE-dependent gene expression but causes metabolic adaptations lowering tissue O_2 consumption.[220] Conditional inactivation of PHD2 in mice is sufficient to activate a subset of HIF target genes, including erythropoietin, leading to striking increases in red blood cell production.[221] Tuning of the normoxic setpoint when the cells are exposed to modest changes in O_2 ambience seems to happen physiologically perhaps as an adaptive response. Comprehension of the pathways involved in such process should help us employ pharmacological and/or genetic approaches to therapeutically adjust the normoxic setpoint on an as needed basis. For example, moderate hypoxia is known to be a robust cue to initiate the angiogenic response. One can reap the angiogenic benefits of that knowledge by adopting therapeutic approaches that would lead to suppression of PHD function resulting in HIF stabilization and HRE-dependent transactivation. Indeed, this approach is being explored for wound therapies.

TISSUE OXYGENATION AND WOUND THERAPY

HIF PHD-directed wound therapeutics

The PHD inhibitor FG-4497 readily stabilizes HIF-1α and subsequently drives the expression downstream of HIF target genes. FG-4497 is helpful in colitis perhaps by benefiting wound healing at the site of inflammation.[222] ECM is predominantly collagen, and the imino acids (Pro and HyPro) comprise 25% of collagen residues. The final step in collagen degradation is catalyzed by prolidase, the obligate peptidase for imidodipeptides with Pro and HyPro in the carboxyl terminus. Defective wound healing in patients with inherited prolidase deficiency is associated with histologic features of angiopathy, suggesting that prolidase may play a role in angiogenesis. Recently it has been demonstrated that prolidase inhibits PHD activity to induce HRE-dependent transactivation and facilitate angiogenic signaling.[223] HIF-specific PHD inhibitors are being tried out for their efficacy in treating wounds. It is likely that such approaches to pharmacologically stabilize HIF will facilitate responses such as generation of angiogenic factors. Whether that response translates to functionally successful angiogenesis and improvements in wound closure will depend on whether other fundamental prerequisites such as a threshold level of tissue oxygenation is present to fuel the healing process. This is of particular concern for ischemic wounds that suffer from extreme chronic hypoxia. If hypoxia alone would have been sufficient to heal, all ischemic wounds would have undergone rapid healing. Clinical observation is exactly the opposite. The key here is to couple hypoxia-response signaling with conditions such as appropriate tissue oxygenation that could sustain the healing process. PHD inhibitors alone are not likely to yield favorable outcomes in extremely hypoxic wounds. Furthermore, it is important to note in this context that PHD inhibition may stabilize HIF but does not guarantee transcriptional function. Co-substrate and cofactor requirements for Fe(II), ascorbate, and the Krebs cycle intermediate 2-OG, and inducible changes in the cellular abundance of three closely related HIF prolyl hydroxylases (PHD1–3) provide additional interfaces with cellular O_2 status that may be important in regulating the oxygen-sensitive signal. Although under conditions of acute hypoxia PHD inactivation supports tissue survival, recently it has been demonstrated that under conditions of chronic hypoxia PHD overactivation is necessary as a survival response.[224] Chronic ischemic tissue overactivates all three isoforms of PHD to survive.[224] The merit of PHD inhibition for the treatment of ischemic wounds involving chronic hypoxia warrants reconsideration in this new light.

First and foremost it needs to be borne in mind that the overarching goal of oxygen therapy should be to correct wound hypoxia. While to some extent hyperoxia may be well tolerated by tissues, it would be prudent to avoid extreme hyperoxia.[225] Although oxygen toxicity may not be imminently overt, an overdose of O_2 is likely to trigger molecular responses such as cell cycle arrest and epigenetic modifications,[226,227] which would oppose healing. Second, approaches to keep a wound oxygenated over a longer period of time, as opposed to a few hours usually targeted in HBO therapy, should prove to be beneficial. In response to HBO, there is no sustained change in tissue O_2 tension much beyond the period of treatment.[228]

The most fundamental factors in wound care are fluid management, temperature management, pain control, increased arterial O_2 tension, the use of appropriate sterile techniques, and administration of prophylactic antibiotics.[229] In addition, numerous cellular and molecular players are required to act in concert to successfully execute wound healing.[230,231] While examining the efficacy of O_2 therapy in wound healing, it is critically important to recognize that O_2 cannot act in isolation. Oxygen therapy may be only expected to benefit in those cases where the remaining essential players are functional and hypoxia is the only rate-limiting factor. Thus, oxygen therapy is generally recommended as an adjunct to other forms of wound care.[232,233]

HBO

HBO therapy represents an effective approach to bolster tissue O_2 levels[5] and has been found to benefit wound healing under specific conditions.[234–238] Importantly, HBO may potentially work synergistically with growth factors such as PDGF to improve the outcomes of ischemic wounds.[20] Because PDGF requires O_2-derived H_2O_2 for successful function, this finding is not surprising.[239] HBO causes sharp elevation in tissue pO_2.[240,241] The administration of two atmospheres of 100% O_2 for 2 hours may raise tissue pO_2 by 10–20-folds[242,243] over the values under basal room air conditions. This systemic approach to oxygenate tissues seems to offer some unique potential advantages. HBO may increase bone marrow NO in vivo thereby increasing the release of EPC into circulation. EPC mobilization into circulation is triggered by hyperoxia through induction of bone marrow NO with resulting enhancement in ischemic limb perfusion and wound healing.[244–246] HBO may also increase NO levels in perivascular tissues via stimulation of NOS. Exposures to 2.0 and 2.8 ATA O_2 stimulated neuronal (type I) NOS

(nNOS) and significantly increased steady-state NO concentration, but the mechanism for enzyme activation differed at each partial pressure. Enzyme activation at 2.0 ATA O_2 appeared to be due to an altered cellular redox state. Exposure to 2.8 ATA O_2, but not 2.0 ATA O_2, increased nNOS activity by enhancing nNOS association with calmodulin.[247] Thus, dosing does seem to matter in HBO therapy. Yet, in the clinics HBO is applied in a standard format to all patients regardless of their individual needs. Could this be an important factor in explaining the less than satisfactory results that HBO is generally thought to have produced in clinical settings?[248] When a flat dose of oxygen is provided to all wound patients, it is possible that the specific dose applied is successful in oxygenating the pockets of extreme hypoxia in some wounds. In these cases, beneficial outcomes should be expected to follow. In the same vein it may be hypothesized that for some other cases, the dose applied is excessive compared with the need of the wound. In these wound with pockets of more moderate hypoxia, the same dose of HBO may be excessive negating the beneficial effects of hypoxia. This is of outstanding interest because excessive oxygen is known to cause growth arrest and accelerate cellular senescence.[249–251]

Because the ability to handle oxygen toxicity is dependent on the expression of genes encoding antioxidant proteins,[252–259] it is possible that in some patients predisposed to oxidative stress the massive increase in tissue pO_2 following HBO results in molecular responses such as growth arrest,[212–214,260] which may not manifest overt signs of oxygen toxicity but does resist wound healing. Another consideration in this regard would be the observation that a large fraction of chronic wound patients suffer from malnutrition.[261–265] Such individuals are also known to be predisposed to oxidative stress and are limited in their ability to fend against oxygen toxicity.[266–268] It is therefore reasonable to propose that chronic wound patients suffering from malnutrition are predisposed to HBO-induced oxidative stress. Taken together, such hypotheses would explain the inconsistent outcomes reported following HBO treatment[269–272] and call for HBO dosing regimens where physicians would prescribe the target wound pO_2. This approach would be consistent with the emerging concept of personalized healthcare[273] and would require the design of new HBO devices fitted with the capability of real-time mapping of wound O_2 tension as can be made possible via technologies such as electron paramagnetic resonance spectroscopy.[274,275]

Topical oxygen

Studies reported during the last 5 years renew interest in examining the significance of topical approaches to oxygenate cutaneous wounds as adjunctive therapy.[1,14,18,276,277] Topically applied O_2 gas is able to modestly increase the pO_2 of the superficial wound tissue.[277] In cases where hypoxia of the superficial wound tissue is a key limitation, topical oxygenation should prove to be helpful. Encouraging results obtained from the use of topical O_2 gas in both clinical[1,18] as well as preclinical[277] settings warrant serious consideration of this approach. Recently, perfluorocarbon droplets encapsulated in aqueous continuous phase has been used as topical O_2 emulsion to treat experimental wounds. Results from this double-blind in vivo study demonstrate that topical approaches to oxygenate the wound significantly enhance the rate of epithelialization of partial-thickness excisional wounds and second-degree burns. Whether the emulsion was able to increase wound tissue pO_2 was not examined, however.[276] Epithelial wound healing is improved by transdermal sustained-delivery treatment with 100% O_2.[14] A recent clinical study testing the effects of topical O_2 gas application on chronic wound presented clinically reports significant improvement in wound size. Interestingly, topical oxygen treatment was associated with higher VEGF expression in the wound edge tissue.[18] Pure O_2 is known to induce VEGF.[15,63,219] Findings of the study testing the effects of topical oxygen gas on chronic wounds are consistent with previous findings suggesting that topical treatment may induce wound angiogenesis.[278] Randomized clinical trials testing the effects of topical oxygenation on wound outcomes are warranted.

HBO and topical oxygen approaches have several contrasting features. The systemic effects of HBO, both favorable as well as unfavorable, may not be expected with topical oxygen. Topical oxygenation can only modestly increase tissue pO_2[277] and cannot match the large increases in tissue pO_2 typically noted in response to HBO.[242,243] If the goal is to correct hypoxia of the superficial tissue, topical approaches should be helpful. However, if the goal is to achieve larger supraphysiological levels of tissue pO_2, HBO would represent the approach of choice. An advantage of topical approaches is that they are portable and therefore applicable in a field or home setting. The cost advantage of topical oxygenation over HBO is another practical consideration.[276,279,280]

SUMMARY

The etiology of chronic ischemic wounds is generally multifactorial of which hypoxia is a common factor in most cases. Primarily based on the tumor literature, hypoxia is generally viewed as being angiogenic. This is true with the condition that hypoxia be acute and mild–modest in magnitude. Extreme hypoxia, as commonly noted in problem wounds, is not compatible with life or tissue repair. Adequate wound tissue oxygenation is required but may not be sufficient to favorably influence healing outcomes. Success in wound care depends on a personalized health care approach. The key lies in our ability to specifically identify the key limitations of a given wound and in developing a multifaceted strategy to address those limitations. In considering approaches to oxygenate the wound tissue, it is important to recognize that both too little as well as too much may impede the healing process. Oxygen dosing based on the specific need of a wound therefore seems prudent. Therapeutic approaches targeting the oxygen sensing and redox signaling pathways are promising as well. Investment in bringing such capabilities to clinical practice should yield lucrative returns.

ACKNOWLEDGMENT

Supported by NIH awards RO1 HL073087, GM 077185, and GM 069589 to CKS.

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Section III

Case Studies

Advanced Oxygen Therapy Inc.

TopicalWound® OXYGEN

Topical Wound Oxygen (TWO_2) used with Standard Best Practice Wound Care on Recalcitrant Lower Extremity Ulcers

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Abstract

Chronic foot ulcers remain notoriously difficult to heal despite the use of standard best practice wound care. Wound care literature is replete identifying local tissue hypoxia as an impairment to wound healing. We have found that the addition of topical oxygen to recalcitrant pedal ulcers enhances their healing. The authors present a series of four patients with five foot ulcers that have been recalcitrant to multiple treatment modalities greater than four weeks . All patients were diabetic and all ulcers closed.

Introduction

Oxygen has an integral role in wound healing. Physiologically oxygen is involved with the enzymatic production of collagen and is therefore important for angiogenesis and granulation tissue. Adequate delivery of oxygen to the ulcer cells is therefore vital for healing.

Methods

Patients selected for presentation had diabetic foot ulcers recalcitrant to standard best practice wound care four weeks or greater. The Topical Wound Oxygen System, manufactured by AOTI Ltd, Ireland was used for 90 consecutive minutes daily 7 days /week. The Topical Wound Oxygen System delivered 100% oxygen to the wound bed utilizing pressure cycles between 5 and 50mbar.

During the treatment period, all patients received current standard best practice wound care techniques including infection control; debridement of devitalized tissue either enzymatically or via sharp debridement; offloading or compression therapy; plus the addition of topical pressurized oxygen therapy. Foot dressings were not disturbed and oxygen permeable dressings such as Kling and gauze were used.

Results

All patients were male, average age 57, achieved closure on 5 previously non-healing pedal ulcers. These ulcers were recalcitrant to standard practice wound care for an average of 15.6 months. The average ulcer time to closure using topical oxygen was 3.4 months (1month-6 month) and average number of treatments to closure at 45 (10-105).

Prior to treatment the non healing ulcers averaged 3.13 cm2 (0.08-4.90 cm2) in area. The ulcers either extended deep to the subcutaneous tissue (3/5), deep to the bone (1/5) or deep to the tendon (1/5).

Patient 1- DEHISED SURGICAL WOUND

Patient 59 year old nursing home male patient with history of PVD, CAD, hyperlipidemia, HTN, PTSD and foot Osteomyelitis seen for care of non healing foot ulcer for 18 months. The ulcer was located at the lateral border of the right foot. Patient had partial amputation of his 5th metatarsal to remove the infected bone. One month later when the ulcer was free of infection, Apligraft was applied to the surgical site as it had dehisced. The graft failed and subsequently a graft jacket was tried just one month after the Apligraft application. It also failed despite standard wound care. Topical oxygen was then attempted on this 2 cm x 0.4cm deep to the subcutaneous tissue. After 4 weeks the ulcer was closed. The patient unfortunately passed away 3 months later from an acute MI.

Patient 2 - MEDICAL PATIENT WITH HX of HEPATITIS C AND SICKLE CELL

Patient 52 year old actively employed male patient with history of sickle cell trait, hepatitis C, leukocytosis and substance abuse was seen for care of non healing foot ulcer located on the dorsum of the left foot, present for thirty six months. The ulcer began as the result of an injection that contained dexamethasone phosphate and was used to treat painful second metatarsal phalangeal joint bursitis. The ulcer became deep to tendon. Despite standard wound care for two years that included the VAC the ulcer would not close. Topical oxygen was then attempted on this ulcer 3.8 cm x1.3 cm deep to tendon. After 6 months the ulcer was closed. Since closure, now three years, there has been no breakdown of this previously ulcerated area.

Patient 3- DEHISED SURGICAL WOUND

56 y/o male with a past medical history of osteoarthritis, insulin dependent diabetic , substance abuse (cocaine, EtOH , opioid), hypertension presents to the emergency room with a surgical dehisced wound present four weeks after a triple arthrodesis procedure. Patient was admitted to the hospital from the ER with significant redness and swelling to LEFT foot from noncompliance. The patient's bandage became wet while on his boat and subsequent the surgical site dehisced. After consulting Infectious Disease the patient was placed on IV Vanco 1g q12hrs, for 6 weeks for a MRSA infection. After four weeks of standard based wound care, the previously infected and dehisced wound was not healing. The wound measured 6 cm x 3.5 cm deep to bone. Topical Oxygen was added, and after 4 months of therapy, the ulcer was closed. Since closure, now one month, there has been no breakdown of this previously ulcerated area.

Patient 4- MEDICAL PATIENT WITH PROSTATE CANCER

65 y/o male presents to the clinic with a right great toe inter phalangeal joint ulcer present for 11 months measuring 0.1 cm x 0.8 cm and deep to the dermal layer and granular with a hyperkeratotic rim. Patient had a past medical history of prostate cancer, osteoarthritis, and insulin dependent diabetes. Patient states that the ulcer originally occurred when walking in a pair of sandals and he noticed blood on his socks. After addition of topical oxygen to standard based wound care the ulcer closed in one month.

Additionally, the patient had a twelve month old heel ulcer measuring 1.3 cm x .5 cm deep to the subcutaneous layer. The wound base was granular with a hyperkeratosis rim. No peri-wound erythema, no edema, no drainage, no malodor noted. After addition of topical oxygen to standard based wound care the ulcer was closed in four months.

After five months of standard based wound care the previously infected dehisced wound heel and grit toe ulcer was not healing. The wound measured 6 cm x 3.5 cm deep to bone. After 4 months of standard based wound care the ulcer was closed. Since closure, now one month, there has been no breakdown of this previously ulcerated area.

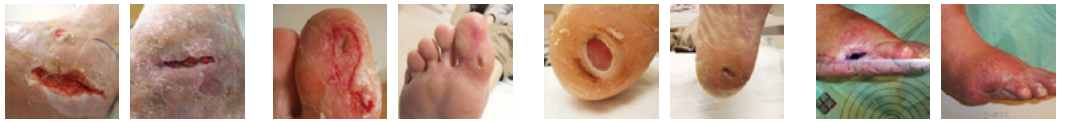
Patient Data

Patient	Topical O_2 Treatment (months)	Ulcer Duration (months)	HBA1C (4.2-5.3)	Albumin (3.5-5.3)	ABI	Side (R,L)	Gender (M,F)	Age (Years)
		x=	x=	x=				
S	1	18	5.4	1.6	0.8	R	M	59
K	4	1	6.8	3.6	0.8	L	M	56
M1	1	11	9.2	3.9	0.6	L	M	60
M2	5	12	9.2	3.9	0.6	L	M	60
T	6	36	5.8	4.2	1.17	L	M	52

TOPICAL OXYGEN TREATMENT: ULCER SIZE (CM.) VS. TIME (MONTHS)

Time (month)	Patient 1	Patient 2	Patient 3	Patient 4 Toe	Patient 4 Heel
Initial	2.0 x .4 x SQ	3.8 x 1.3 x T	6 x 3.5 x B	.1 x .8 x SQ	1.3 x .5 x SQ
1	closed	3.0 x 1.3 x T	5 x 1.5 x B	.1 x closed	.5 x .5 x SQ
2		3.0 x 1.3 x SQ	4.8 x 1.1 x SQ		1 x 1 x SQ
3		2.5 x .5 x SQ	3.5 x .5 x D		.6 x .7 x D
4		.9 x .3 x D	closed		.7 x .5 x SQ
5		closed			closed

B= deep to bone; D= deep to dermis; T=deep to tendon; SQ= deep to subcutaneous



A Retrospective Review of Topical Wound Oxygen As An Adjunct Treatment in Healing Chronic Ulcerations

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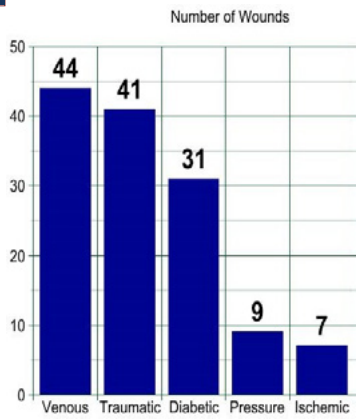
INTRODUCTION

Topical Wound Oxygen is a modality that utilizes cyclical topical oxygen pressure with non-contact compression that rivals the benefits of hyperbaric oxygen without the hassle and complications. Topical Wound Oxygen has been utilized in challenging chronic ulcerations with or without other advanced therapies. This study is a retrospective chart review analyzing healing among patients with chronic ulcerations of various etiologies in an urban veteran population who were treated with Topical Wound Oxygen.

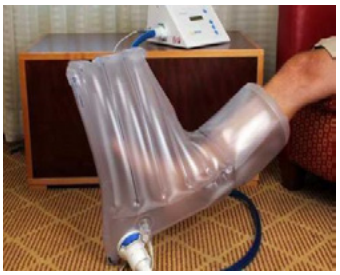
METHODS

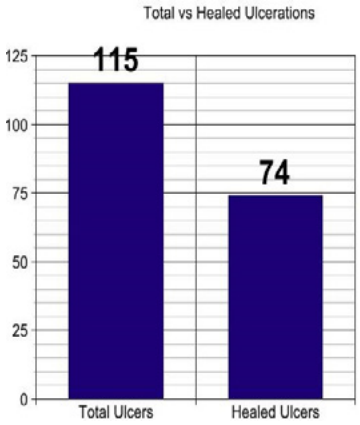
This is a two year retrospective review which includes 71 patients with a total of 115 wounds from a Veterans Affairs hospital that were prescribed cyclical pressurized Topical Wound Oxygen (TWO2)* for use at home. Patients with wounds of venous/lymphatic insufficiency were most commonly prescribed the therapy (44), followed by surgical or traumatic wounds (41), diabetic (31), pressure (9) and ischemic ulcers (7). Most patients had concurrent peripheral vascular disease ranging from mild to severe, with or without vascular intervention. Eighteen patients were excluded: eight were lost to follow-up, four discontinued due to development of gangrene to the extremity, four that received amputations with surgical closure, one patient expired, and one discontinued due to sickle cell disease.

* Advanced Oxygen Therapy Inc. (AOTI)



TWO2 Homecare Therapy





RESULTS

Of 115 ulcers under treatment of topical wound oxygen in two years, 64.4% (74) achieved complete closure. This retrospective analysis improves the overall closure rate from our previously submitted studies, which demonstrated a healing rate of 56% and 44% closure. The majority of open ulcerations were concurrently treated with advanced tissue products, and venous or arterial pumps during the treatment course, due to the extreme complexity of their comorbidities. Of the ulcerations that have not reached full closure, the majority have decreased in size since initiation of topical oxygen. To note, although not specifically tested during this study, a number of patients (5) stated they had significant pain relief with the usage of Topical Wound Oxygen device.

CONCLUSION

The continuation of this large retrospective analysis continues to demonstrate that cyclical pressurized Topical Wound Oxygen is a valuable adjunct modality in the treatment of chronic ulcerations. Results show an encouraging closure rate and noted wound improvement on these recalcitrant ulcerations. Further larger Randomized Controlled Trials are in progress to validate these outcomes, including a multicenter, multinational, double-blinded, placebo controlled RCT (NCT02326337) that has just concluded recruitment.

Abstract No. EPP124: EWMA 2018, Krakow Poland, May 9 - 11

Advanced Oxygen Therapy Inc.

TopicalWound® OXYGEN

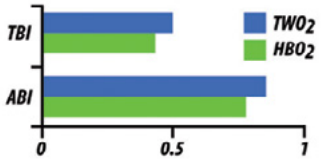
Hyperbaric and Topical Wound Oxygen: A Comparative Study

Francis Derk, DPM • STVHCS: Chief Podiatry Services • UTHSC: Assistant Clinical Professor

Two similar cases of Partial First Ray Amputations secondary to Osteomyelitis and soft tissue infection were compared. Negative pressure was used in both cases set at 125 mm of HG for approximately 3 weeks , changed 3 times weekly, and started day 1 in conjunction with HBO_2 or TWO_2 modalities. Topical wound care and off loading consisted of wet to dry dressings and Cam boots respectively. The TWO_2 patient lived over 65 miles from the nearest HBO_2 facility and could not afford the costs of transportation.

Safety: Both wounds were debrided, titrated to antibiotics per the C&S,and normal WBCs were attained prior to initiation of therapies as listed below.

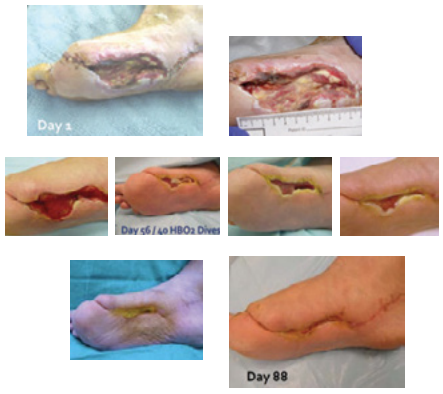
Patient Data	HBO_2	TWO_2
Age	52	64
DM	13 yrs	21 yrs
Hx of amputation	x2	x1
PMH	DM, HTN, Hep C, Cirrhosis	DM, HTN, Obesity, Kidney Dx
Smoking	20 pack yrs	Negative
ABI/TBI	.85 / .45	.90 / .52



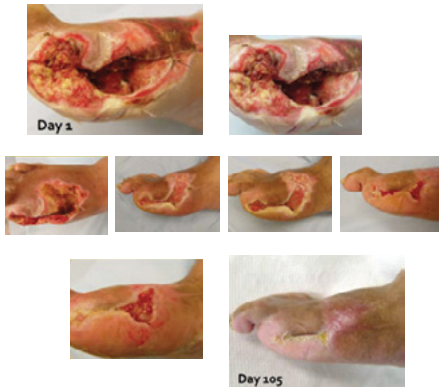
Results:

- **The TWO_2 wound took 17 days longer to heal**
- **TWO_2 costs were less expensive**
- **TWO_2 was utilized to full closure vs HBO_2 whichwas limited to 40 dives (day 56 and not fully healed)**
- **The HBO_2 wound apart from 40 dives required 32 additional days of conventional wound healing to closure**
- **No baro-trauma or complications were incurred**

Hyperbaric Oxygen (HBO_2)



Topical Wound Oxygen (TWO_2)



MODALITY	Wound Size/ Depth	Negative pressure	O2 Therapy	Healing time	Cost
HBO2	11.2 x 4.8 cm depth 2.7 cm	17 days	40 dives (56 days)	88 days	$23,160
TWO2	11.9 x 5.4 cm depth 3.1 cm	23 days	210 applications (105 days)	105 days	$11,445

HBO_2 and TWO_2 are both viable options in healing large open wounds. TWO_2 has been shown to be cost effective, and a comparative healing modality . TWO_2 is an excellent, alternative choice to HBO_2 especially when considering financial resources, limitations with health, availability, and convenience (home usage).

Advanced Oxygen Therapy Inc.

TopicalWound® OXYGEN

Treatment of a Chronic Stage IV Pressure Ulcer using Topical Wound Oxygen (TWO_2) Therapy

Anku, Comfort RN, Dr. Christian Frye[2]
[1] Post Inn Village, Toronto, Canada, [2] AOTI Ltd, Galway, Ireland

Introduction

Chronic wounds are frequent, difficult to treat and show high rates of complications. We examined the clinical efficacy of a unique pressurized topical oxygen therapy (TWO_2) device in a long term care setting in Canada on a 67 y/o male patient with a stage IV pressure ulcer.

Method

The patient was treated daily with TWO_2 therapy for 90 minutes. Prior to each treatment, the patients wound dressings were removed and the wound bed was irrigated with a normal saline solution. After each TWO_2 treatment, the wound was treated with Silversorb and Betadine then redressed with standard gauze dressing. The TWO_2 device delivered humidified medical grade oxygen at a constant pressure of 30 mbar. The wound care coordinator performed weekly wound assessments including photos to document the wound area, volume and changes in each from the previous assessment.

Results

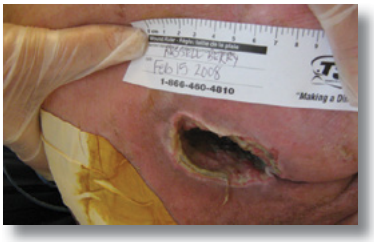
Initial wound measurements indicated the ulcer had an area of 31.2 cm2 with a volume of 109.2 cm3. Tissue was noted to be very necrotic and the peri-wound was macerated. After one week of treatment, the wound area and volume had increased slightly, however the physician noted that the maceration had improved. Week 2 measurements showed a decrease in both area and volume with significant granulation. By week 3, the wound was 95% covered with granulation and it was noted the peri-wound was less friable. Wound area had decreased by 43% and the volume by 41% and dressings were now being done with Dermagen packing. The patient was hospitalized after 6 weeks of therapy for an unrelated condition. At that time, his wound area had decreased to 4.55 cm2 and volume to 11.38 cm3. TWO_2 therapy was discontinued during the hospitalization. TWO_2 resumed one month later; with an area of 5.28 cm2 and volume of 12.5 cm3. After 2 additional weeks of therapy, the wound had 100% closure.

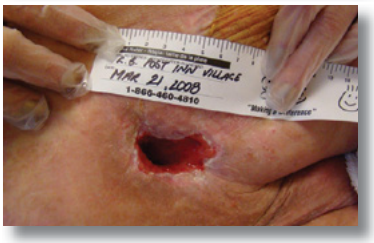
Observations:

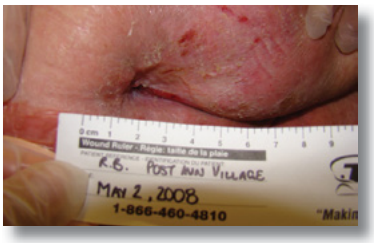
1. TWO_2 improves local tissue perfusion
2. TWO_2 softens necrotic tissue and enhances debridement
3. TWO_2 eliminates maceration
4. TWO_2 reduces nursing intervention time

Conclusion

Patients with severe chronic wounds benefit from the treatment with TWO_2 and show remarkable wound closure rates.







Advanced Oxygen Therapy Inc.

two2 MULTI-MODALITY OXYGEN THERAPY

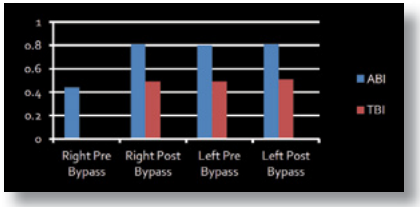
The Use of Topical Wound Oxygen (TWO_2) in a Complicated Acute Venous Embolism and Thrombosis of the Lower Extremity

Francis Derk, DPM , CDR USN STVHCS: Chief Podiatry Services UTHSC: Assistant Clinical Professor

A 66 yr/o Male underwent a Femoral-Popliteal Bypass for a non healing right dorsal foot wound. Eight days following the procedure, the patient developed right lower extremity thrombosis resulting in the formation of deep sub dermal eschars. The patient was admitted for leuckocytosis and wound management.

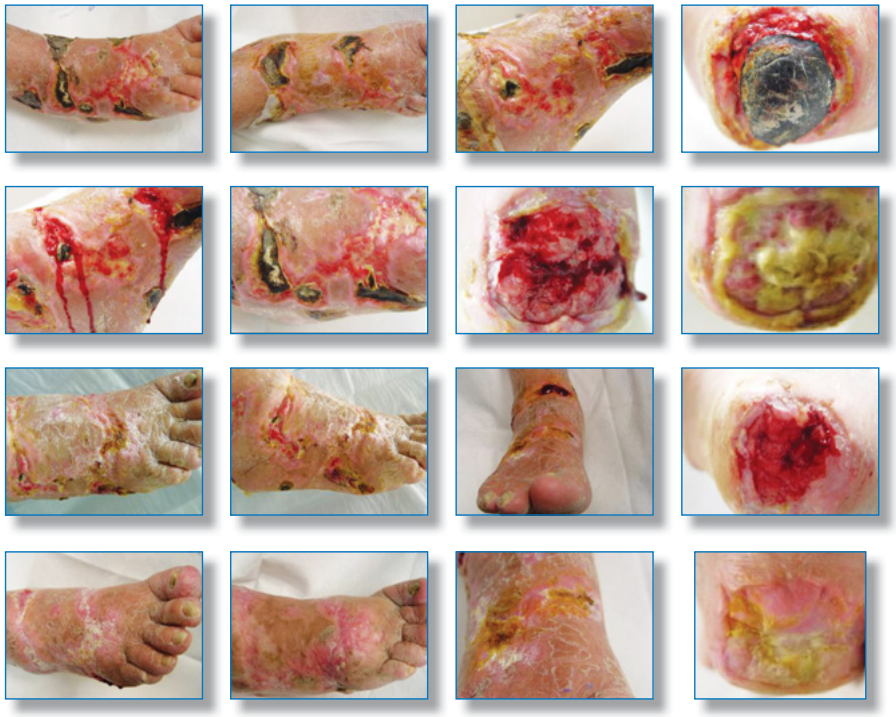
PMH: PVD, HTN, Obstructive Chronic Bronchitis

Smoking: 1 PPD / 40 pack year hx



Right	Pre Bypass	Post Bypass S/P 2 months
ABI	.44	.81
TBI	0.0	.49

Admission: Hospital Course: x 1 week (WBC 14.2)
IV antibiotics: Zosyn (3.375 gms IV q 6 hrs) x 6 days
C&S: Staph aureus (negative MRSA)
X-rays: negative
Discharge: Amoxicillin (250 gms qid x 14 days)
WBC: 6.7 / Sed Rate 20 / CRP 1.5



TIMELINE

S/P 3 weeks TWO_2 Treatments
90 min/BID following Discharge

BID Santyl Dressings multiple wounds dorsum, medial and lateral ankles, heel *various stages and levels

S/P 6 weeks TWO_2 Treatments 90 min/BID

Anticoagulation: 7.5 mg Warfarin x 30 days

S/P 10 weeks TWO_2 Treatments
90 min/BID

Anticoagulation: 5.0 mg Warfarin x 30 days

S/P 16 weeks TWO_2 Treatments
90 min/BID
Fem-Pop bypass: patent

Conclusion: TWO_2 in conjunction with Santyl dressings proved to be very effective in this very unique case study and a viable option in treatment of ischemic wounds.

Advanced Oxygen Therapy Inc.

TopicalWound® OXYGEN

Topical Wound Oxygen As An Adjunct Treatment in Healing Chronic Ulcerations

Sarah J Park, DPM • Aamir Mahmood, DPM • Patrick J Sanchez, DPM • Jake G Ruff, DPM • Anna Tien, DPM • Justin Goldsmith, DPM • Andrea Seat, DPM • Michael Czurylo, DPM
Laith Shaman, DPM • Matthew Garoufalis, DPM, FASPS, FACFOAM

Background

Topical Wound Oxygen (TWO_2) is a multi-modality utilizing oxygen at high pressures with a topical application along with non-contact compression. TWO_2 has been utilized during all phases of healing in challenging chronic ulcerations with or without other advanced therapies. This retrospective chart review evaluated healing among patients with chronic ulcerations of varied etiologies who were treated with TWO_2 in conjunction with standard and advanced treatments. This analysis is a continuation of previously published data incorporating 15 additional patients allowing for further investigation into previously presented results.

Methods

In this study, 52 patients with a total of 92 wounds from one hospital were prescribed TWO_2. Patients with wounds of venous/lymphatic insufficiency were most commonly prescribed TWO_2 (40), followed by surgical or traumatic wounds (20), diabetic (18), pressure (9) and ischemic ulcers (5). The patients with concurrent PVD had a range from mild-severe with or without vascular intervention. Eight patients were excluded: 6 lost to follow-up, 1 due to dry gangrene and 1 who was prescribed TWO_2 following elective 1st ray surgery with sickle cell disease.

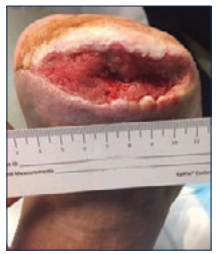
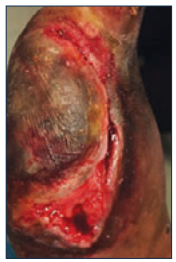
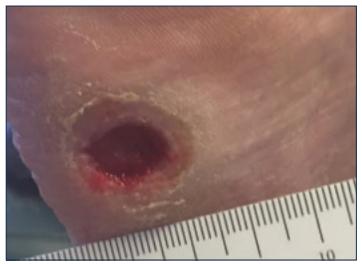
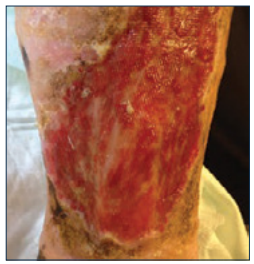
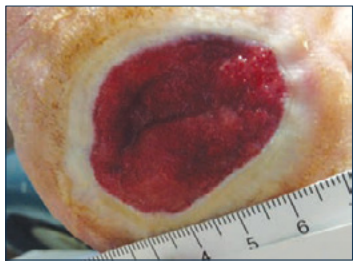
Results

Upon data collection of the 84 ulcers, 46 have completely epithelialized, representing a 54.76% closure rate. This additional analysis improves the overall closure rate from our previously published study, which demonstrated a healing rate of 43.9%. With the exception of 8 healed ulcers, the remaining wounds utilized adjunctive treatment including tissue products, venous pumps or arterial pumps during the treatment course due to the extreme complexity of the patients. Of the ulcerations that have not reached full closure, the majority have decreased in size since initiation of TWO_2.

Conclusions

This continuation of a large retrospective analysis continues to demonstrate that TWO_2 is a valuable adjunct modality in the treatment of chronic ulcerations. Results show an encouraging closure rate and noted wound improvement on these recalcitrant ulcerations.

Topical Wound Oxygen can be utilized with various types of wounds: surgically created wounds, diabetic foot ulcers, venous leg ulcers.

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Section IV

Professional Society Recommendations & Guidelines

USE OF OXYGEN THERAPIES IN WOUND HEALING

FOCUS ON TOPICAL AND HYPERBARIC OXYGEN TREATMENT

JWC journal of wound care

A JOINT DOCUMENT

WoundsAustralia

EWMA

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The document is supported by an unrestricted educational grant from: ActiMaris, AOTI, Inotec AMD, Oxy-Care and SastoMed.

This document has been created in close cooperation with the European Underwater Baromedical Society (EUBS) and the European Committee for Hyperbaric Medicine (ECHM)..

This article should be referenced as: Gottrup F, Dissemond J, Baines et al. Use of oxygen therapies in wound healing, with special focus on topical and hyperbaric oxygen treatment. J Wound Care, 2017; 26(5), Suppl, S1–S42.

Published on behalf of EWMA by MA Healthcare Ltd.
Editor: Rachel Webb
Designer: Lindsey Butlin
Publisher: Anthony Kerr
Published by: MA Healthcare Ltd, St Jude's Church, Dulwich Road, London, SE24 0PB, UK
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Contents

Abbreviations

- ATA: Absolute atmosphere
- CI: Confidence interval
- CCD: Conventional compression dressings
- CDO: Continuous delivery of non-pressurised oxygen
- CMS: Centers for Medicare & Medicaid Services
- CW: Chronic wound
- DFU: Diabetic foot ulcer
- EWMA: European Wound Management Association
- FGF-2: Fibroblast growth factor-2
- HBOT: Hyperbaric oxygen therapy
- HR: Hazard ratio
- HRQoL: Health-related quality of life
- HTA: Health technology assessment
- IL: Interleukin
- IWGDF: International Working Group on Diabetic Foot
- MRSA: Meticillin-resistant *Staphylococcus aureus*
- NICE: National Institute for Health and Care Excellence
- NOX-2: NADPH oxidase of phagocytes
- NPWT: Negative pressure wounds therapy
- NNT: Number Needed to Treat
- NO: Nitric oxide
- pO_2: partial pressure of O_2
- PAOD: Peripheral arterial occlusive disease
- PVP-1: Povidone iodine
- PU: Pressure ulcer
- QoL: Quality-of-life
- RCTs: Randomised controlled trials
- RR: Relative risk

- ROS: Reactive oxygen species
- RVU: Refractory non-healing venous ulcer
- SR: systematic reviews
- SW: Sloughy wound
- SOS: Super-oxidised solution
- TCOM: Transcutaneous oximetry
- THO: Topical 'hyperbaric' oxygen
- TNF-alpha: Tumour necrosis factor-alpha
- TO: Topical oxygen
- TOT: topical oxygen therapy
- UHMS: Undersea and Hyperbaric Medical Society
- VEGF: Vascular endothelial growth factor
- VLU: Venous leg ulcer

1. Introduction

Among other things wound healing requires restoration of macro- and microcirculation as essential conditions for healing.[1,2] One of the most 'immediate' requirements is oxygen, which is critically important for reconstruction of new vessels and connective tissue and to enable competent resistance to infection.

Sustained oxygen is also vital for the healing of patients with non-healing wounds. This has been proven for wounds associated with peripheral arterial occlusive disease (PAOD) and diabetic foot ulcers (DFUs).[3]

Non-healing wounds are a significant problem in health-care systems worldwide. In the industrialised world almost 1–1.5% of the population will have a non-healing wound at any one time. Furthermore, wound management is expensive; in Europe it is expected that wound management accounts for 2–4% of health-care budgets. These figures will probably rise along with an increase in the elderly and diabetic populations.[4–7]

Oxygen therapy is a general term that covers hyperbaric oxygen therapy (HBOT) and topical oxygen therapy (TOT) among other treatments. HBOT has been known for many years and is well established as essential conditions for healing. Therefore, in this document HBOT is presented as the synopsis of mechanisms of action, clinical evidence and current recommendations of internationally recognised hyperbaric organisations. In recent years new therapeutic approaches based on TOT have been developed to support wound healing. Due to its relative novelty and small number of clinical studies compared with HBOT, the description of several methods classified as TOT are presented in more detail with description of most, including still ongoing, studies. The imbalance in the volume of description between the two treatment methods, we provide, must be carefully judged by the reader with special attention to the grade of evidence and level of recommendations. In future, the relation between TOT and HBOT, with possible synergistic action, must be taken into account when planning further studies.

Aim, objectives and scope

The overall aim of this document is to highlight the present knowledge with regard to the use of oxygen therapies in the care and treatment of wounds of different aetiologies, which fail to progress through an orderly and timely sequence of repair. In this document, these types of wounds are defined as 'non-healing'.[8]

Excluded from this document are animal and cellular models, acute wounds, such as surgical/trauma wounds and burns. The distribution of supplementary systemic oxygen at barometric pressure in connection with surgery is not covered by this document.

We provide an overview of the treatment options, as well as assessments of the best available evidence on their respective results. In addition the document will go into detail with specific

aspects and current discussions regarding the use of oxygen in wound healing including:

- The role of oxygen and hypoxia in the wound healing process
- Patient perspectives of oxygen treatment
- Cost-effectiveness aspects of oxygen therapies
- What remains controversial with suggestions for future actions.

In line with other similar documents published by the European Wound Management Association (EWMA) during recent years the document structure is inspired by the different elements that are usually included in the health technology assessment (HTA) approach. Thus, it is not a traditional position document that discusses different treatment strategies, when to use which product, or assesses one product against another, but rather a holistic picture of the current practice and reality of the use of oxygen therapies in wound healing.

Structure and content

The document is presented in nine chapters. Chapters 4–7, which present the main content and analysis, follow the same structure of: introduction, main content including level of evidence, conclusion and recommendations.

- Chapter 1: Introduction to the document including its aim, objectives and scope as well as a short summary of its structure
- Chapter 2: Presents the methodology and terminology used in the document
- Chapter 3: Introduces and discusses the role of molecular oxygen in living tissue in general and in wound healing processes specifically
- Chapter 4: Presents and discusses TOT
- Chapter 5: Presents and discusses HBOT
- Chapter 6: Focuses on patient perspectives of oxygen treatment including health-related quality of life (HRQoL) and patient education
- Chapter 7: Presents considerations regarding economics and cost-efficiency of TOT and HBOT
- Chapter 8: Conclusions of the document
- Chapter 9: Provides a brief look at expected new developments over the next few years in the area of oxygen therapies and wound healing.

2. Methodology and terminology

This document originates from requests and expressions of interest in a document focused on the role and use of oxygen in wound healing by various EWMA stakeholders.

On the basis of a literature search conducted in PubMed by the EWMA secretariat, as well as input from key EWMA stakeholders, a short description of the document aim, objectives and scope was developed during the second quarter of 2015. This basic document outline was then used over the next six months to identify the specialists, who constitute the author group.

In addition to current and former members of the EWMA Council the author group includes a representative of Wounds Australia (www.woundsaustralia.com.au), a representative of the European Underwater and Baromedical Society (http://www.eubs.org/) and the European Committee for Hyperbaric Medicine (http://www.echm.org/), as well as individual and independent specialists from Europe and the US.

Each author has taken responsibility for the elaboration of the first draft of a whole or part of a chapter. It has been the obligation of each author to search and investigate the relevant literature.

The opinions stated in this document have been reached by a consensus of the author group, weighing their professional opinions based on their individual research and that of their peers as well as their own clinical experience.

Assessment of availability and levels of evidence

Throughout this document the GRADE classification of levels of evidence will be used to assess the evidence level of the different oxygen therapies described. An overview of the GRADE classification system is available in Appendix A of this document.

Oxygen therapies are similar to wound care in general in being characterised by the limited existence of high-level evidence regarding the documented effect of most of the therapies used. Many are used because in practice they offer good treatment results. However, high-level evidence is lacking due to the absence of systematic reviews (SR), randomised control trials (RCTs), or other evidence at a higher level than cohort or case-studies.

In spite of the generalised absence of higher level evidence this paper will make recommendations on the basis of the data available.

Table 1 refers to the terminology we have used in this document.[9–13]

Table 1. Terminology

Term	Definition
Biofilm	A coherent cluster of bacterial cells imbedded in a biopolymer matrix, which, compared with planktonic cells, have increased tolerance to antimicrobials and resists the antimicrobial properties of host defence[9]
Colonisation	Microbial multiplication in or on the wound without an overt immunological host reaction[9]
Contamination	Microbial ingress into the wound without growth and division[10]
Endpoint	The occurrence of a disease, symptom, sign, or laboratory abnormality that constitutes the target outcomes of a clinical trial[11]
Hyperbaric oxygen therapy (HBOT)	Exposing the whole body to pressure exceeding 1 absolute atmosphere (ATA) when patient breathes pure oxygen, which is transferred with circulation to all body tissues
Hypoxia	Inappropriately low availability of molecular oxygen
Infection	Invasion and multiplication of microorganisms in body tissues, evoking an inflammatory response (systemic and/or local) and causing local signs of inflammation, tissue destruction, and fever.[12] It is perhaps worth noting that definitions of wound infection vary,[13] but that diagnosis is based on clinical signs and symptoms[9]
Outcome	Documentation of the effectiveness of health-care services and the end results of patient care
Reactive oxygen species (ROS)	Reactive molecules containing oxygen
Resource use	The total amount of resources actually consumed, compared against the amount of resources planned for a specific process[12]
Topical oxygen therapy (TOT)	The administration of oxygen applied topically over injured tissue by either continuous delivery or pressurised systems
Wound cleansing	Removing harmful substances (for example, microorganisms, cell debris, and soiling, from the wound, so that the healing process is not delayed/hindered or to reduce the risk of infection[10]

3. Role of molecular oxygen in wound healing

Sufficient availability of molecular oxygen (O_2) is essential for proper wound healing and it has long been recognised that development of non-healing wounds is more frequent when partial pressure of O_2 (pO_2) in the wound is below a critical hypoxic threshold level. Hypoxia may result when consumption of O_2 supersedes the delivery of O_2. Poor blood perfusion is traditionally associated with reduced supply of O_2 leading to hypoxia in wounds, which can lead to deficient healing, but the depletion of O_2 resulting from the biological activities within the wound may also contribute significantly to the availability of O_2.[1,14]

Oxygen consumption during wound healing

In general, basic need for energy is mainly covered by consumption of O_2 during aerobic respiration. However, a reduction of O_2, due to its role in the production of reactive oxygen species (ROS) during the respiratory burst of activated phagocytes is an essential part of the initial inflammatory response to tissue damage. Furthermore, O_2 is the most immediate requirement for wound healing in order to reestablish new vessels and connective tissue. O_2 consumption by the NADPH oxidase of phagocytes (NOX-2) is necessary for phagocytes to produce adequate amounts of lactate to activate transcription factors that promote the development of angiogenesis factors. The reconstruction of connective tissue is also influenced by the amount of O_2 available for consumption during maturation of collagen fibres and appropriate fibroblast proliferation. Furthermore, O_2 consumption supports a competent host-response to infection due to the requirement of O_2 for generation of suitable amounts of antimicrobial ROS by phagocytes.[1,14]

Oxygen supply in wounds

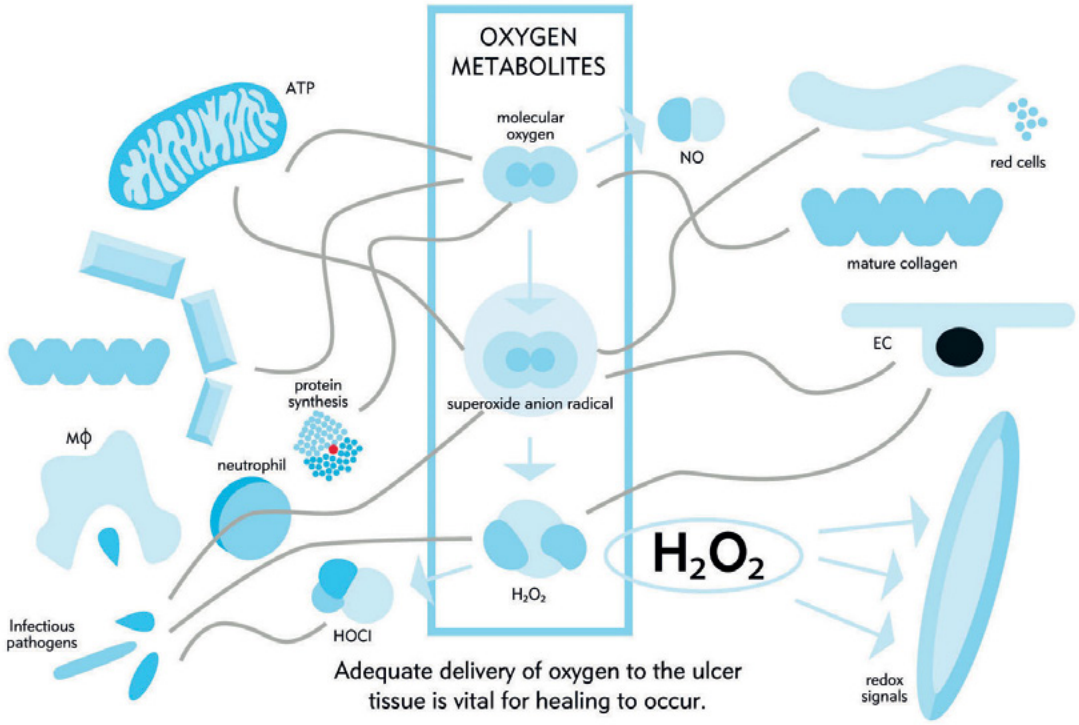
O_2 delivery in wounds predominately depends on pO_2 in the adjacent tissue and the circulating blood.[15] Thus, oedema, the injured microcirculation and contraction of the vessels in traumatised tissue may prevent an adequate supply of O_2. In addition, poor blood circulation may also inhibit the distribution of O_2 in to the wound. Other barriers to appropriate O_2 supply include diffusive constraints due to oedema and O_2 consumption by bacterial biofilm. Also of note, the high metabolic activity present in healing wounds will reduce overall levels of tissue oxygen content.

Extra oxygen consumption in wounds with a chronic infection

Neutrophils are the predominating phagocytes in humans and increased O_2 consumption is a typical response to a vast variety of stimuli including infectious Gram-negative or Gram-positive bacteria, fungi, and even sterile tissue damages.[16–19] The main reason for the extra O_2 consumption is the activation of the phagocytic NADPH-oxidase in order to produce ROS and the ability of NOX-2 to reduce O_2 has been subject to several studies demonstrating the ability to deplete O_2 even when levels are already low.

If the attracted neutrophils manage to successfully

Fig 1. The role of oxygen in wound healing



Adapted from C.K. Sen. Wound healing essentials: let there be oxygen. Wound Repair & Regeneration 2009; 17:1–18

clear the tissue of microbial intruders and pro-inflammatory debris, their work ceases, resulting in reduced accumulation and decreased consumption of O_2, with progression towards resolution and healing of the injury. However, if the bacteria are able to resist the attacking neutrophils, as seen when bacteria are organised in biofilm, a situation occurs where the bacterial biofilm attracts activated neutrophils that deplete the microenvironment of O_2 for ROS formation without eradication of the bacteria. Likewise, failure to resolve the tissue damage and clear debris in the wound may cause an accumulation of neutrophils that advance the consumption of O_2 to an extent where proper wound healing is delayed and even prevented.

In chronic wounds evidence for bacterial existence in biofilm is increasing and infiltration of

Table 2. Methods for measuring levels of O_2 in wounds

Method	Reference
Near-infrared spectroscopy	31–33
Pulse oximetry	34
Tissue oxygen tension	35
Transcutaneous oxygen tension measurement	36

neutrophils surrounding *Pseudomonas aeruginosa* and *Staphylococcus aureus* organised in biofilm may occur.[20,21] In addition, experimental infection with *Pseudomonas aeruginosa* biofilm has demonstrated increased accumulation of neutrophils in mouse wounds.[22] However, an actual demonstration of accelerated hypoxia caused by the activity of the summoned neutrophils in chronic wounds infected with biofilm remains to be done, but indirect observation points to a possible significant contribution to hypoxia by activated neutrophils. These observations include steep gradients of O_2 down to levels of hypoxia in wounds of diabetic mice with wounds infected with *Pseudomonas aeruginosa* biofilm.[23] Such steep oxygen gradients have also been demonstrated in fresh debridement specimens from infected human wounds.[23]

Furthermore, among the bacterial genes that were expressed during the biofilm infection of the wound were genes associated with low levels of O_2 and the hypoxia-stress response, indicating that the host response restricts the availability of O_2.[23] The ability of neutrophils to significantly restrict the availability of O_2 is known from other biofilm-associated infections with hypoxia.[18] In particular, the accelerated O_2 depletion by neutrophils is the predominating mechanism of the O_2 consumption in freshly expectorated sputum samples from patients with biofilm-associated chronic pneumonia.[18,24] Likewise, neutrophils are the major consumer of O_2 when exposed to *Pseudomonas aeruginosa* biofilm *in vitro*.[16] This further indicates that O_2 depletion is a general response by neutrophils to biofilm. As in infected wounds, the freshly expectorated sputum from patients with pneumonia contains steep gradients of O_2[18,25] and bacterial gene expression from chronic pneumonia corresponds to microenvironments where the neutrophils are restricting the availability of O_2. Further evidence for O_2 depletion by neutrophils during infection, comes from the upregulation of genes related to hypoxia in *Staphylococcus aureus* from the synovial fluid of patients with prosthetic joint infection,[26] which is typically characterised by intense accumulation of activated neutrophils.[27]

Examination of the ecology in chronic wounds may also reveal the existence of zones with O_2 depletion. Accordingly, the very high frequency of facultative aerobic and strictly anaerobic bacterial species from chronic wounds[28,29] may be regarded as surrogate biomarkers for sustained hypoxia in chronic wounds. Similarly, the biochemical composition of wound fluid may contain information about the physiology of the wound. In this way, the higher concentration of lactate in wound fluid than in serum[30] indicates ongoing anaerobic glycolysis, which is linked to neutrophil activity and metabolism at hypoxic conditions.

Thus, activated neutrophils may contribute to hypoxia and if the source of activation persists the neutrophils may prolong hypoxia, which may prevent the wound in the inflammatory phase entering the resolving and regenerating phase. In this respect, monitoring levels of wound O_2 may provide guidance to whether wounds with poor healing are associated with a lack of O_2 and if supplemental O_2 may result in re-oxygenation and improved healing of wounds. Several methods for measuring levels of O_2 in wounds have been successfully applied and should be used to estimate level of oxygenation and efficacy of the therapeutic effect (Table 2).[31–36] It should be pointed out that these methods measure local hypoxia but do not allow us to estimate the effect on the level of neutrophils.

Conclusion

Even though hypoxia acts as an initial physiological signal to promote wound healing, prolonged hypoxia may maintain pro-inflammatory conditions and prevent resolution and restoration of wounds. Thus, ongoing hypoxia induced by chronic infections, including enhanced O_2 consumption by activated neutrophils, may impede proper healing of the wound.

Recommendation

Measurement of local tissue oxygenation before and during hyperbaric oxygenation may assist health professionals in identification of patients likely to benefit from HBOT. However, all O_2 therapies, including local O_2 supply or delivery enhancement by haemoglobin, will benefit from the knowledge of the O_2 levels in the proximity of the wound. Measurement of pO_2 near the wound, so called transcutaneous oximetry (TCOM), is currently approved as the best surrogate for oxygenation of the wound bed. This measurement strongly depends on several factors, including local perfusion, temperature reactivity, and O_2 outflow through the skin layers.[37]

The predictive value of TCOM has been mathematically validated for diabetic extremity ulcers with good prediction of the failure rate when taking a TCOM measurement while breathing oxygen at pressure.

4. Topical oxygen therapies

Despite almost 50 years of clinical use, the subject of TOT for non-healing wounds remains controversial.[38–42] TOT can be defined as the administration of oxygen applied topically over injured tissue by either continuous delivery or pressurised systems. The availability to the wound tissue of topically applied higher pO_2 reverses localised hypoxia.[43] This causes both the direct killing of anaerobic bacteria and an enhancement of leukocyte function to address all other pathogens.[44,45] Once the inflammatory cascade subsides, the high availability of oxygen molecules in the wound tissue helps to upregulate angiogenic growth factors like vascular endothelial growth factor (VEGF) and fibroblast growth factor-2 (FGF-2).[45] This results in the prolific structured growth of new blood vessels and the stimulation of collagen synthesis by enhancing fibroblast activity.[46–48] These factors combined result in better wound bed granulation, strong collagen tissue formation, and wound closure.[46,47,49]

Background

The first report of TOT was published in 1969[41] wherein this therapy was called 'topical hyperbaric oxygen'. However, the term 'hyperbaric' as used in that paper was misleading and incorrect as currently used. Using specially constructed topical chambers on 52 patients with wounds of varying aetiologies, pure humidified oxygen was delivered under a constant pressure of 22mmHg; oxygen was applied continuously for 4–12 hours a day. Although uncontrolled by current standards, success was noted in the majority of cases with only six reported failures with an average healing time of three weeks in those treated with pressurised oxygen. It was found that wounds subjected to O_2 therapy at ambient pressures improved, but more slowly than those under pressure.[41] In the first RCT of topical 'hyperbaric' oxygen (THO) treatment, a total of only 28 patients were allocated to THO (n=12) and control (n=16) groups. All patients were admitted to the hospital for debridement, local dressings, intravenous antibiotics, and bedrest. The intervention group received THO in only four daily 90 minute sessions using a leg chamber providing humidified 100% oxygen under cycled pressures between 0 and 30mmHg. During the 14-day study period both groups experienced progressive reductions in the size of their DFUs. Not surprisingly, there were no significant differences in wound area reduction between the two groups. The obvious (and fatal) flaws in this study were the small numbers of patients treated and the very limited time period under study. There was simply insufficient power to detect any differences in treatments should any exist at only two weeks. The standard time frames that are currently employed for such DFU wound healing studies are 12-week treatment periods. Nonetheless, this study is often quoted as 'evidence' that THO is ineffective in promoting healing of foot ulcers.[50] In the following years there were inconsistent results in case series and reviews suggesting the putative benefits of administering oxygen topically to chronic wounds.[45,47,51–54]

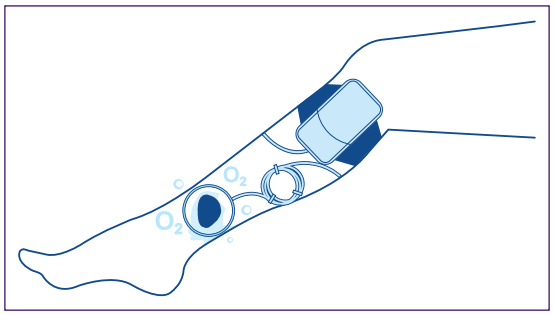
A subsequent non-randomised study sought to evaluate the healing benefits of both HBO and topical oxygen (TO) in a group of 57 patients with

a variety of chronic wounds.[45] Using standardised protocols for both therapies, healing outcomes were assessed at 14 weeks. Although they found no statistically significant change in wound volume reduction in the HBO group after this treatment period, the 25 wounds subjected to TOT showed a significant 57% reduction after 14 weeks of treatment (4 days each week). Additionally, wound edge tissue biopsies were taken to assess VEGF gene expression at baseline and at treatment end. Comparing VEGF expression at the final time point to the baseline measurement, those wounds treated with TO achieved a significant induction of VEGF expression, higher in those wounds where wound healing/volume reduction occurred. The overall difference in VEGF gene expression for HBO treated patients was not found to be statistically significant, although there was indeed an increase noted for most patients.[45] This study provides further evidence that treatment with topical oxygen can have a beneficial effect towards the healing of chronic wounds

Continuous delivery of non-pressurised oxygen

This category of devices apply topical continuous delivery of non-pressurised (normobaric) oxygen (CDO) through small cannulas or thin tubes to essentially occlusive wound dressings. Small portable battery-powered oxygen generators (extraction units) supply a continuous flow of pure oxygen to the wounds 24 hours a day.[3] The wound dressings are typically changed weekly and the oxygen generators are generally replaced after one to two weeks of continuous use.

The interim results of the RCT of the TransCuO$_2$



Continuous delivery of non-pressurised oxygen

CDO device showed that wound closure at 12 weeks was not significantly associated with treatment per the protocol, active 11 (52.3%), sham 8 (38.1%), [relative risk (RR) 1.38; 95% confidence interval (CI): 0.7, 2.7), p=0.54].[55] However, in the recently published results of the completed RCT a significantly higher proportion of people healed in the active arm compared with the sham arm (46% versus 22%, p=0.02). This relative effect became greater in more chronic wounds (42.5% versus 13.5%, p=0.006). Patients randomised to the active device also experienced

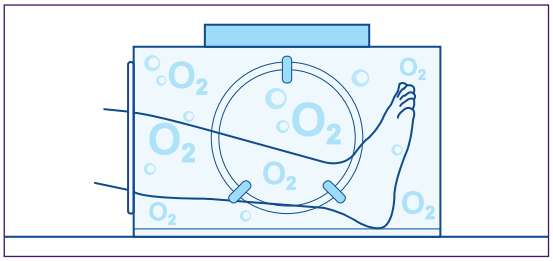
Table 3: Technologies available for distribution of topical oxygen in wound healing

Technologies available for distribution of topical oxygen in wound healing
Continuous delivery of non-pressurised oxygen (CDO)
Low constant pressure oxygen in a contained chamber
Higher cyclical pressure oxygen
Oxygen release through dressing or gel
Oxygen transfer
Application of oxygen species

significantly faster rates of closure relative to the sham ($p<0.001$). Unfortunately, this was only a per protocol analysis of the first 50 patients in each arm to complete the 12-week trial.[56]

Despite several small case studies indicating beneficial healing for chronic wounds,[57,58] results for the Epiflo device multicentre RCT have yet to be published in any journal. Nonetheless, information available on clinicaltrials.gov indicates that wound closure at 12 weeks was not statistically significantly associated with treatment per the protocol active 55.7%, sham 50.8% with 61 patients in each group.[59] A prior single centre randomised study of 17 DFU patients followed for four weeks indicated that the TO group achieved an average wound size reduction of 87% compared with 46% in the standard of care group ($p<0.05$).[60] While tissue and wound sample cellular and cytokine level changes were noted, these patients were not followed to complete healing and the sample size was too small to be widely generalisable.

The Natrox CDO device has been marketed for several years with posters and presentations indicating positive results in a variety of wounds. A small published case series on the treatment of venous leg ulcers (VLUs) indicated positive results towards healing and a reduction in pain scores during the treatment periods.[61] A recent small, single-centre, randomised non-placebo controlled trial of 20 patients with chronic DFUs compared this device with standard care alone over 8 weeks.[62] They found a significantly increased healing rate (wound area reduction) in those treated with the topical oxygen device compared with baseline at week 8 ($p<0.001$), but no such increased difference was noted in the control group ($p<0.262$). While all superficial ulcers healed in both groups, the TOT group seemed to show a more beneficial effect in more advanced ulcers. While published data is not yet available, a large RCT using this device is currently in progress to further determine its efficacy in healing chronic DFUs.



Oxygen delivery in a contained chamber

Low constant pressure oxygen in a contained chamber

The lower constant pressure devices include such devices as the O_2 Boot or OxyCare. In this approach oxygen is provided in a simple plastic chamber/boot that is placed around the extremity with the ulcer. Constant pressure is then maintained within the chamber up to 35mmHg. There are numerous studies that have been conducted on these types of devices over the last four decades that have ostensibly shown good clinical efficacy. However, the majority of these studies have consisted of case series or uncontrolled trials.[45] The one very poorly conducted RCT that used a similar device has been previously discussed.[50] Unfortunately, this study is often cited as evidence of the ineffectiveness of TO despite its being underpowered and of too short of a duration. This outcome is not surprising considering the fact that the therapy arm only received two treatments each week (four total treatments) with the O_2 therapy devices used.

Higher cyclical pressure oxygen

The Topical Wound Oxygen (TWO_2) system differs from other devices in that it applies a higher topical O_2 pressure between 5mmHg and 50mmHg, in a cyclical pressure waveform, combined with humidification. The benefit of this approach is that the higher pressure gradient results in O_2 molecules

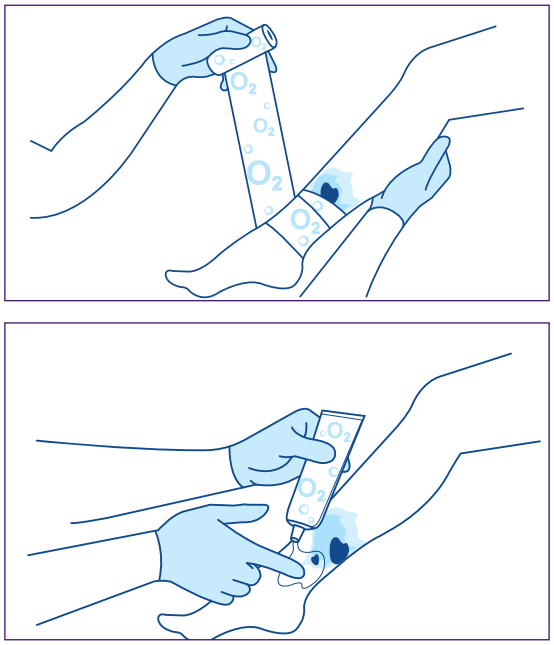
diffusing deeper into the hypoxic wound tissue and enhances multiple molecular and enzymatic functions.[46,63] The cyclical pressure applied with TWO_2 of between 5mmHg and 50mmHg creates sequential non-contact compression of the limb that helps to reduce peripheral oedema and stimulates wound site perfusion further.[48,64] Several prospective clinical studies have been conducted using this device on both VLUs and DFUs. One non-randomised parallel arm study of 83 patients was conducted on VLUs to measure the effect of TWO_2 compared with conventional compression dressings (CCD) on wound healing using the primary endpoint of the proportion of ulcers healed at 12 weeks.[48] At 12 weeks, 80% of TWO_2 managed ulcers were completely healed compared with 35% of the CCD-managed ulcers. Median time to full healing was 45 days in the TWO_2 arm and 182 days in CCD arm. Unfortunately, there was a good deal of selection bias pertaining to treatment allocation in this study. These same authors later conducted another comparative study that similarly investigated the efficacy of TWO_2 versus CCD in the management of refractory non-healing venous ulcers (RVUs) with a duration of at least two years.[64] This study was also non-randomised and allotment to treatment arm was primarily based on patient preference. A total of 132 patients were enrolled with 67 patients (mean age: 69 years) using TWO_2 and 65 patients (mean age: 68 years) with CCDs for 12 weeks or until full healing. At 12 weeks 76% of the TWO_2 managed ulcers had completely healed, compared with 46% of the CCD-managed ulcers with a median time to full healing of 57 days and 107 days, respectively. Interestingly, in those patients with meticillin-resistant *Staphylococcus aureus* (MRSA) colonised ulcers, MRSA elimination occurred in 46% of patients managed with TWO_2 and 0% of patients managed with CCD. Another prospective non-blinded, non-randomised study was conducted to examine the clinical efficacy of TWO_2 therapy in healing patients with severe DFUs referred to a community wound care clinic in Canada.[65] Patients were simply allocated to the TO if a unit was available or were otherwise treated with advanced moist wound therapy. At 12 weeks 82.4% of the ulcers in the TWO_2 therapy arm and 45.5% in the standard care arm (control) healed completely. Median time to complete healing was of 56 days in the TWO_2 therapy arm and 93 days in the control standard care arm. An ongoing study is currently enrolling subjects into a 220 patient multinational, multicentre, prospective, randomised, double blinded, placebo-controlled trial to evaluate the efficacy of TWO_2 in the treatment of chronic DFUs. The study's inclusion criterion allows for non-healing DFUs up to Stage 2D in the University of Texas Classification of Diabetic Foot Ulcers, defined as wounds penetrating to tendon or capsule with infection and ischaemia. It includes a two-week run-in period with best standard of care to flush out wounds that would heal with this alone and a 12-month follow-up to assess recurrence. With a standardised primary outcome of the incidence of complete wound closure at 12 weeks, this trial should not only address the need for TOT, but it should also make its results comparable with other advanced wound care therapies including systemic HBOT.[66]

Oxygen release through dressings or gels

Different kinds of products are available, either using the release of pure O_2 embedded in the dressing or releasing O_2 generated by a biochemical reaction in a hydrogel. In the O_2 containing dressings, pure O_2 is embedded, such as in vesicles, and released after the dressing is liquefied by the wound exudate. Continuous O_2 release dressings can be used as secondary dressing and release O_2 for up to six days. In order to optimise conditions for delivery at the wound, debridement and cleansing should be carried out at regular intervals before the dressings are applied.

In hydrogel dressings an increased concentration of dissolved O_2 is obtained via a chemical or

biochemical reaction. These occlusive dressings make use of the reactivity of 0.3% hydrogen peroxide, which is converted to water and dissolved O_2. This can diffuse via a permeable separator to the wound bed. In contrast, another product consists of two separate components must be applied together to activate the biochemical process. One component contains a hydrogel sheet containing glucose and a low-concentration gel matrix with less than 0.04% of iodide ions, and a second component sheet containing glucose oxidase. The glucose oxidase incorporated in the second gel sheet catalyses the oxidation of (beta)-D-glucose to D-gluconic acid and hydrogen peroxide in the presence of O_2. The hydrogen peroxide released as a result is thought to diffuse through the dressing and either oxidises iodide ions to free iodine and O_2 or, if it reaches the wound surface, is metabolised to water and O_2. Iodine has a beneficial antimicrobial effect within the gel and should help to prevent the proliferation of microorganisms at the wound–dressing interface, while the dissolved O_2 is believed to create beneficial effects within the wound.[3]



Oxygen release through dressings or gels

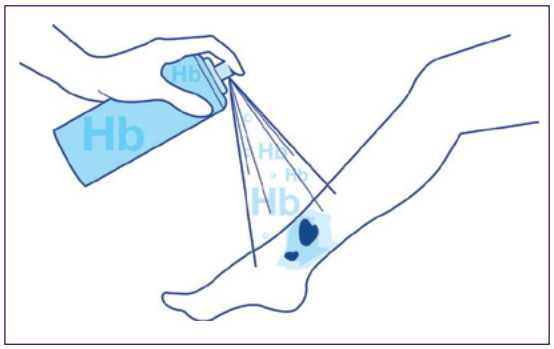
Several case study reports demonstrate improvements in the healing of different wound types.[67,68] As an example, in a non-controlled multicentre case series of 51 patients the dressing was tested over a six-week period in wounds with various aetiologies and a mean duration of 25.8 months. The results showed six wounds healed fully, 37 were judged to have improved, seven remained static and one deteriorated.[69] *In vitro* experiments have shown that such dressings are capable of significantly increasing O_2 levels in wounds.[70] Further evidence of its beneficial impacts on wound healing was generated by using these dressings on burn patients treating larger donor site wounds in comparison with standard care.[71] Moreover the oxygenating hydrogel dressings, which release O_2 and different levels of iodine into the wounds, were tested in different *in vitro* tests against various target organisms. It was shown that the dressings were significantly more effective against a broad spectrum of microorganisms including biofilm than controls.[72,73]

Oxygen transfer

Haemoglobin as an O_2 carrier is another approach to topical wound treatment. Haemoglobin augments transport of O_2 by means of facilitated delivery.[74] The mode of action of this approach is based solely on the physical effect of facilitated delivery, and not on a pharmacological or metabolic effect. In wound treatment, the haemoglobin spray should be applied in addition to standard therapy. The spray can be used concomitantly with most existing treatment regimens.[3] In a pilot study the O_2 saturation of ulcer tissue was measured in five patients with chronic leg ulcers before application and 5 and 20 minutes after application using photoacoustic tomography. The average O_2 saturation showed

a significant increase up to 5mm depth from 56.4% before to 69% after 5 minutes and 78.8% after 20 minutes following a single application of haemoglobin spray. The authors conclude that the application of topical haemoglobin spray leads to an increase in O_2 saturation *in vivo* in patients with chronic leg ulcers.[75]

The authors of an RCT compared the application of the haemoglobin spray versus a sham product as add-on to best practice wound care over 13 weeks. In each treatment group there were 36 patients. In contrast with the control group, where no wound size reductions were observed, the patients treated with the complementary haemoglobin spray demonstrated a significant wound size reduction of 53%.[76] The clinical effects of a haemoglobin spray were also observed in a multicentre observational evaluation of 17 patients with 20 chronic DFUs. In 14 of the 18 wounds that completed the evaluation over a four-week period a mean reduction in wound size of 53.8% was observed. After 12 weeks 20% had healed, 53% were progressing towards healing, 20% increased in size and 7% were slow to heal.[77] In a case series of 11 patients with pressure ulcers (PUs) who were treated with haemoglobin spray for three months, nine wounds healed and two demonstrated reduced wound-size. From ten patients with pain at baseline, nine were pain-free by week 8. A rapid elimination of slough was observed in all patients.[78] In another set of recently collected data cohorts, sequential patients were recruited prospectively from patients with DFUs, chronic wounds (CWs), and sloughy wounds (SWs). The number of patients recruited to each cohort was 20, 50 and 100 respectively. As control group, data from clinical notes of an equal number of patients were collected retrospectively. These were selected sequentially by date in the notes without reported as matching to prospective cases. The DFU cohort was treated in a hospital setting and the CW/SW cohorts were treated in primary care. All three cohorts shared the inclusion criterion of a wound that failed to heal defined as a <40% reduction in area in the previous four weeks. In the DFU cohort the mean wound size reduction was greater in the haemoglobin spray group at week 4 (–63% versus –21%), week 16 (–91% versus –43%) and week 28 (–95% versus –63%). At week 28 follow-up, 15/20 patients in the haemoglobin spray cohort had complete healing compared with 8/20 in the control cohort. The CW cohort reported mean wound size reductions of –73% in the haemoglobin spray group compared with –12% in the control group at 4 weeks. The benefit persisted at 8 weeks (–87% versus –14%) and the final 26 week follow-up (–89% versus –75%). Altogether 45/50 patients had complete healing at the final 26-week follow-up compared with 19/50 in the control group. The SW cohort results were reported in a more limited fashion. At week 8 follow-up there was a mean wound size reduction of –93% in the haemoglobin spray group compared with –32% in the control group. At week six complete wound closure was observed for 65/100 patients in the haemoglobin spray group and 37/100 patients in the control group.[79,80]



Oxygen transfer

Based on the published evidence and positive clinical outcomes regarding the efficacy of haemoglobin spray practical-oriented clinical algorithms have been established for this kind of treatment both by

the German-speaking D.A.CH.-(Germany, Austria, Switzerland) region[81] and in England.[82]

Application of oxygen species

Another therapeutic approach using topically applied O_2 in wound treatment is based on the fact that ROS can be used in antimicrobial treatment and perhaps as a signalling molecule that support wound healing processes.[79,80] ROS are effective in destroying a broad range of pathogens and also biofilms. Their mode of action is typically the physical destruction of the pathogen's cell-wall integrity and hence they are not linked to the problems of antibiotic resistance, which are related to a range of pharmacological effects. There is an increasing spectrum of products using ROS for antimicrobial and cleansing wound therapy available. A product containing hyperosmotic ionised seawater, ROS, triplet oxygen $^3O_2$ and a high pH-value is thought to reduce wound swelling, inflammation, microbial contamination and to stimulate cellular signalling transduction pathways. It is available as a rinsing solution and a wound gel. The antimicrobial effects are mediated primarily by the singlet O_2.

These effects are regulated by the basic pH value which supports a high concentration of hydroxyl ions, which act as an antioxidant.

In a cohort study conducted in four wound clinics, the clinical efficacy of singlet O_2 solution was

Table 4. Types of topical oxygen devices and therapies currently available

TOT type	Medical devices	Treatment details				
	Company, Product			Treatment location	Moist wound environment	GRADE
Higher cyclical pressure oxygen	Aoti Inc., TWO_2	50mbar to 5mbar cycles;	Pressure low, >1bar Flow rate high Treatment time: 60–90 minutes Treatment frequency: 3–7 days	Open wound in chamber or bag	Possible	Grade 18, (RCT, controlled cohort studies, various case series) positive effect shown
Low constant pressure oxygen in a contained chamber	OxyCare GmbH, O_2TopiCare System	2-5 l/min;<50mbar;	Pressure: low, >>1bar Flow rate: high Treatment time: 60–90 minutes Treatment frequency: 3–7 days	Open wound in chamber or bag	Possible	
	GWR Medical, TO_2	2-5 l/min;<50mbar;	Pressure: low, >1bar Flow rate: high Treatment time: 60–90 minutes Treatment frequency: 3–7 days	Open wound in chamber or bag	Possible	
Continuous delivery of non-pressurised oxygen (CDO)	Ogenix Inc., EpiFLO	Continuous, slow flow of pure oxygen of 3 ml/hr for 15 days through a cannula to blanket the wound.	Pressure: low, <1bar Flow rate: low Treatment time: 24 hours Treatment frequency: 7 days	Occlusive wound dressing	yes	Grade 2C, (1 Interim report on RCT showed no advantage versus sham. Cohort studies, various case series) only weak evidence
	Inotec AMD Ltd., Natrox	Continuous, slow flow of pure oxygen of ~12ml/hour for several days via a thin flexible tube to the Oxygen Delivery System which is in direct contact with the wound surface	Pressure: low, <1bar Flow rate: low Treatment time: 24 hours Treatment frequency: 7 days	Occlusive wound dressing	yes	

evaluated. In 73 patients with critically colonised and/or infected, malodorous wounds, covered with slough/fibrin, or wounds showing inflammation of the periwound skin were included. After 42 days 33% of the wounds in the study had healed, 57% had improved and 10% remained stagnant. All wounds had shown clinical signs and symptoms of critical colonisation and/or infection at day 0, at day 42 the infection was completely eradicated and inflammation was reduced in 60%.[83]

Table 4. Types of topical oxygen devices and therapies currently available

Oxygen release through dressing or gel	OxyBand Technologies Inc., OxyBand	Oxygen release for up to 5 days after contact with moisture within a simple occlusive wound dressing	Pressure: na Flow rate: na Treatment time: 24 hours Treatment frequency: 7 days	Occlusive wound dressing	yes	Grade 2B, (1RCT, cohort studies, various case series) only weak recommendation for oxyzyme by Nice due to lack of efficacy
	AcryMed/ Kimberly Clark, OxygeneSys Continuous	Use as a foam dressing, Oxygen release for up to 5 days when dressing is moistened	Pressure: na Flow rate: na Treatment time: 24 hours Treatment frequency: 7 days	Occlusive wound dressing	yes	
	AcryMed/ Kimberly Clark, OxygeneSys On Demand	Oxygen release for up to 5 days after contact with moisture within a simple occlusive wound dressing	Pressure: na Flow rate: na Treatment time: 24 hours Treatment frequency: 7 days	Occlusive wound dressing	yes	
	Crawford Healthcare Ltd, Oxyzyme	Use as a primary dressing, in early stage wound treatment. Oxygen release when both layers are attached to each other	Pressure: na Flow rate: na Treatment time: 24 hours Treatment frequency: 7 days	×	yes	
Oxygen transfer	SastoMed GmbH, Granulox	Liquid spray with 10% purified haemoglobin, applied as thin layer to the wound bed, and before wound is covered by a non-occlusive dressing, twice weekly up to once daily application depends on wound status	Pressure: na Flow rate: na Treatment time: 24 hours Treatment frequency: 7 days	×	yes	Grade 1B, (1RCT, 1 controlled open label study 3 controlled cohort studies, various case series) positive effect statistically shown, >50,000 treatments in more than 20 countries with no relevant side effects, clear positive benefit risk value
Application of oxygen species	Buchs· Actimaris	wound rinsing solutions and wound gel	Pressure: n.a. Flow rate: n.a. Treatment time: few minutes to hours Treatment frequency: at dressing change	×	yes	Grade 2C, 1 cohort study

Other products contain super-oxidised solution or gel manufactured through the electrolysis of ultra-pure water and NaCl. The active ingredient as source of ROS is hypochlorous acid (HOCl), a major inorganic bactericidal compound of innate immunity.[84] HOCl has been shown to be effective against a broad range of microorganisms either as stabilised neutral or acidic HOCl-solutions.[85] These solutions are intended for use in the cleansing and debridement phase primarily to decrease the microbial load by eliminating pathogenic microorganisms.

In an RCT, a stabilised super-oxidised solutions at neutral to acidic pH was tested for the treatment of 40 patients with postsurgical lesions larger than 5cm^2 in DFUs. The outcome of the use of the SOS was compared with use of povidone iodine as a local medication. Patients were followed-up weekly for six months. The authors were able to demonstrate that the healing rates, time taken for cultures to become negative and duration of antibiotic therapy were significantly shorter in the group treated with super-oxidised solution.[86] The authors claim that the cost of the super-oxidised solutions is lower than standard treatment with a saving of 40% on the total expenditure, especially due to less antibiotic therapy and following surgical procedures. Results are in accordance with findings of other clinical trials performed. Recently, a safety, effectiveness and cost-effectiveness evaluation of stabilised super-oxidised solutions in comparison with povidone iodine (PVP-I) treatments was published.[87] The authors concluded that such solutions are a safe, effective and cost-effective irrigation and cleansing agents and can provide an economical alternative to the other available antimicrobial agents.

Conclusion

The clinical results achieved with these methods indicate possible benefits over standard care alone. As for many other products used in wound care management, the clinical evidence for the efficacy of topical oxygen-based treatment is not homogeneous and ranges from uncontrolled case reports to RCTs with some limitations. Although most of the published data does not meet the highest standards of evidence, it suggests that such adjunctive therapies are easy to handle, safe and may be potentially effective modalities for use in modern strategies of wound care in specific subpopulations. Interesting question about the concomitant action of TOT with other therapeutic procedures, including HBOT, vascular interventions or skin transplantation, still remains unanswered.

Recommendations

There is a limited but expanding evidence base for successful healing after treatment with TO products, especially in a subset of non-healing patients who failed to achieve an adequate healing response in standard treatment settings. Although the authors endorse the adjunctive administration of TO therapies for non-healing chronic wounds, more robust data from multi-centre prospective placebo-controlled trials affirming their clinical efficacy will be required before this promising therapy can be given a stronger recommendation.

5. Hyperbaric oxygen therapy

Beyond the most superficial cell layers, there is supposedly no significant topical absorption of O_2.[47,88] Therefore, for additional O_2 to be delivered to hypoxic tissues, it must be administered systemically—it must be breathed. HBOT involves exposing the whole body to pressure exceeding 1 ATA when a patient breathes pure O_2, which is transferred with circulation to all body tissues. If given at sufficiently high pressure, typically 2.2–2.5ATA, O_2 dissolved in blood plasma diffuses from microcirculation to wound tissues and reverses local hypoxia, which usually exists in the centre of chronic non-healing wounds.[89] Generally speaking, there are two types of hyperbaric chambers used worldwide: mono-place, where patients stay alone within small pressurised vessels filled with O_2, and multi-place, where several patients can be treated at the same time with medical attendant, either nurse or physician, present inside the vessel for direct assistance and support. In Europe, most hyperbaric facilities use multi-place chambers and in the US rates of multi-place and mono-place chambers are approximately the same. While there is an on-going discussion about the differences between those two types of devices, the final dose of treatment, which is pO_2 breathed by the patient, is exactly the same in those two treatment modalities. In chronic wounds treatment HBOT sessions are normally repeated once or twice daily over several weeks. Such intermittent reversion of local hypoxia restores the optimal conditions for regeneration, but in those patients in whom hyperoxic conditions can be created locally during the HBOT the unique effects of hyperoxia per se or regular stimulation with anoxia–hyperoxia status can be observed.

HBOT and wound healing

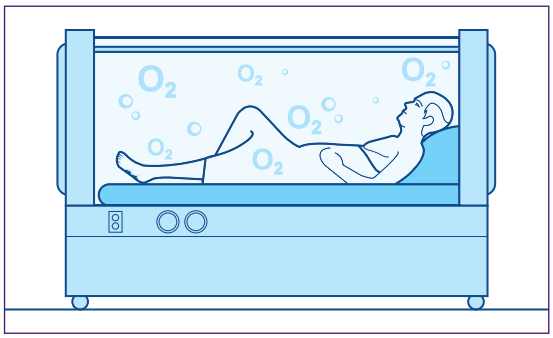
The positive effects of HBOT stem from increasing the tissue O_2 tension and/or pressure within the wound site and have been studied and published in dozens of papers reporting research on humans. The most important actions include:[90]

- Alteration of ischaemic effect
- Reduction of oedema
- Modulation of nitric oxide production
- Modification of growth factors and cytokines effect
- Promotion of cellular proliferation
- Acceleration of collagen deposition
- Stimulation of capillary budding
- Accelerated microbial oxidative killing
- Interference with bacterial proliferation
- Modulation of the immune system response
- Enhancement of O_2 radical scavengers, thereby reducing ischemia reperfusion injury.

An excellent review of use of HBOT in chronic wounds was published by Thackham et al.[92]

HBOT and bacteria

If pO_2 within the wound exceeds the limits for survival of obligate, facultative anaerobes



Mono-place hyperbaric oxygen therapy

and microaerophilic aerobes, the HBOT has a bacteriostatic activity.[93] During *in vitro* experiments, direct bactericidal effect of high enough pO_2 on anaerobic bacteria, i.e. *Clostridium perfringens*, *Bacteroides fragilis*, or *Enterococcus faecalis*, can be observed.[94] But raising the wound O_2 tension increases the capability of leukocytes to kill bacteria and this mechanism explains the indirect antibacterial effect of HBOT on both anaerobic and aerobic strains.[95] Moreover, there is a strong synergistic effect of HBOT with at least some antibiotics, including linezolid, vancomycin, teicoplanin, ciprofloxacin and imipenem.[96–98] We recommend reading the excellent review on HBOT as an anti-infective agent by Cimşit.[99]

HBOT and inflammatory reactions

The anti-inflammatory effects of HBOT have been shown to be mediated by a decrease tumour necrosis factor (TNF)-alpha, interleukin (IL) IL-1beta and IL-8.[100,101] This effect is relatively weak and short acting, which means that it cannot replace the potential use of pharmacological agents to attenuate inflammatory reactions if necessary and that HBOT sessions should be repeated in order to keep that effect.

HBOT and stem cells

Stem cells are mobilised by the HBOT and this effect is observed after a single HBOT session gradually increasing until approximately 20 sessions.[102]

HBOT and genetics

Interestingly, HBOT modifies gene expressions, this has been noted for genes encoding the IL-8 and ANG expression.[101,103] This effect is seen after ending the series of HBOT sessions, when one can observe that healing processes are still persistent for at least several weeks after completing the HBOT.

Monitoring of local oxygenation

The clear TCOM cut-offs for different types of wounds have been established identifying that failure of HBOT is highly probable if TCOM measured at pressure of 2.5ATA while breathing O_2 near the session is lower than 20, 50, 50 or 100mmHg for arterial trauma, musculocutaneous flaps, arterial ulcers or diabetic foot lesion, respectively.[104,105] Other measurement, including near-infrared reflectance spectroscopy or laser Doppler flowmetry and imaging give additional data on oxygenation or microcirculation, but until now they have not been part of routine clinical measurement.

Clinical evidence

There is clinical evidence that HBOT used as the adjunct therapy in selected cases of different types of non-healing wounds can prevent amputations or enhance wound healing. In fact, in the intention-to-treat analysis during one RCT study, complete healing of the index ulcer was achieved in 52% of patients at 1-year follow-up in the HBOT group versus 29% in the placebo group (p=0.03).[106] Moreover, the addition of HBOT to conventional therapy reduces the average healing time in the short term (up to six weeks) when compared with conventional therapy alone in DFUs [Peto Odds Ratio: 14.25; 95% CI: 7.08–28.68],[107] VLUs [mean difference 33.00%, 95% CI: 18.97–47.03, p<0.00001],[108] mixed arterial and venous wounds [mean difference 61.88%, 95%CI: 41.91–81.85, p<0.00001][108] and recurrent non-healing vasculitic

wounds not responding to immunosuppressive therapy.[109] Treatment with HBOT is also associated with a significant reduction in the risk of major amputations, defined as amputations above the ankle joint [RR: 0.29; 95% CI: 0.19–0.44].[110]

Contraindications, side-effects and safety

There are few contraindications known, but—excepting undrained pneumothorax, which is considered an absolute contraindication unless treated—all of them are relative and temporal, including inability to equilibrate pressure within middle ear, fever, claustrophobia, pregnancy, severe heart insufficiency, uncontrolled asthma or concurrent chemotherapy, which could increase O_2 toxicity.[111] HBOT is generally recognised as a safe procedure and the most often observed side-effects include middle ear barotrauma.[112] Other side-effects, including central nervous system or pulmonary oxygen toxicity, are rare.

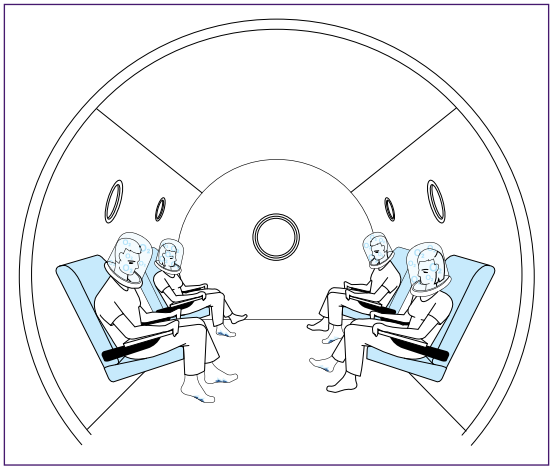
Conclusions

There is evidence that HBOT improves healing by restoration of local hypoxia, exerting an anti-infective effect on both aerobes and anaerobes, decreasing inflammation and oedema, stimulation of angiogenesis and vasculogenesis as well as stem-cells. It should be considered in those cases of non-healing wounds where there is a possibility to restore local hypoxia or induce hyperoxia. Monitoring of the efficacy should be implemented, preferably with TCOM measurements.

Evidence-based recommendations

Based on all available clinical evidence and consensus agreements within the group of internationally recognised experts, the recent tenth European Consensus Conference[113] has issued specific recommendations ranging from 1A–2C for non-healing wounds in different types of wounds (DFUs, VLUs, ischaemic ulcers and systemic inflammatory ulcers) and different populations of patients. An excerpt of these recommendations is included below.

- HBOT is suggested in the treatment of diabetic foot lesion (GRADE 2B)
- We suggest using HBOT in the treatment of ischaemic ulcers (GRADE 2C)
- It would be reasonable to use HBOT in the treatment of selected non-healing wounds secondary to systemic processes (GRADE 2C)
- HBOT is recommended in ischaemic lesions (ulcers or gangrene) without surgically treatable arterial lesions or after vascular surgery:
  - In patients with diabetes, the use of HBOT is recommended in the presence of a chronic critical ischaemia as defined by the European Consensus Conference on Critical Ischemia (see note below), if transcutaneous oxygen pressure readings under hyperbaric conditions (2.5ATA, 100% O_2) are higher than 100mmHg (GRADE 1A)
  - In the arteriosclerotic patient the use of HBOT is recommended in case of a chronic



Multi-place hyperbaric oxygen therapy

critical ischaemia (see note below), if transcutaneous oxygen pressure readings under hyperbaric conditions (2.5ATA, 100% O_2) are higher than 50mmHg (GRADE 2B)

- Note: the chronic critical ischaemia can be recognised when there is: periodical pain, persistent at rest, needing regular analgesic treatment for more than two weeks, or ulceration or gangrene of foot or toes with ankle systolic pressure <50mmHg in the non-diabetic or toe systolic pressure <30mmHg in patients with diabetes[114]

- However, despite the strong agreement on the validity of the criteria listed above to properly select patients for HBOT, the jury acknowledges the fact that not all hyperbaric centres are able to measure transcutaneous oxygen pressure under hyperbaric conditions (2.5 ATA, 100% O_2). Therefore, due to this limitation, we suggest HBOT in DFUs (grade 3 and above of Wagner classification, stage B, grade 3 and above of University of Texas classification) that have failed to respond to adequate basic wound care after 4 weeks (GRADE 2B)

- For the same reason as above, it would be reasonable to use HBOT in delayed healing (chronic), non-diabetic wounds and in recurrent multiple non-healing wounds due to vasculitis (especially those that have not responded to immunosuppressive therapy) (GRADE 2C)

- It is recommended, as the standard of care, that HBOT should always be used as part of a multidisciplinary treatment plan with ongoing wound care on a regular basis and not as a stand-alone therapy (GRADE 1B)

- It is recommended that, before the application of HBOT, standard wound care has been provided during at least a four-week period (including appropriate debridement, vascular screening for significant peripheral arterial disease and/or local wound hypoxia, adequate offloading and infection management) (GRADE 1C)

- It is recommended that, before the application of HBOT, vascular screening including imaging technique is performed in order to evaluate if any revascularisation procedure is indicated (GRADE 1C)

- The use of TCOM is recommended as the best technique to monitor the local pressure of oxygen and to select patients for HBOT (GRADE 1C)

- It is suggested that therapeutic dose of HBOT (pressure, time and length of treatment course) should be adapted to patient, type of chronic wound and evolution (GRADE 2C)

- It would be reasonable to consider HBOT as part of a multiinterventional approach in the treatment of calciphylaxis (GRADE 2C).

6. Patient perspective of oxygen treatment

This chapter explores the patient's perspective of oxygen therapies. Many patients view O_2 as curative,[115] it is a product they are familiar with and many seek out methods to increase their intake of O_2 with the intent of assisting in their wound healing. The patient's impression of an O_2 delivery method may be influenced by the information and education they receive from health professionals, their own experience of O_2 treatment and the progress of their condition as it impacts on their quality of life. However, there is a paucity of published evidence concerning the patient's perspective in the fields of HBOT, TOT and wound management O_2 introducing products (such as haemoglobin spray). Therefore much of the discussion presented is grounded in and extrapolated from low levels of evidence.

Patient/clinical outcome

Soon and Chen[116] described HRQoL tools as an attempt to capture 'patient important outcomes', although they are designed and used by health professionals. At this time there is no HRQoL tool specific to O_2 therapy for patients with wounds.[117] However, data from a range of currently used HRQoL scores may yield information on the efficacy of O_2 therapies from the patients' subjective perspective.

Prospective outcome data collected from patients with a chronic wound and receiving HBOT[118–120,121] have demonstrated an increase in HRQoL and more specifically a reduction in the level of pain experienced in patients with chronic wounds.[122] Pain has also been noted to be reduced with the use of a topical haemoglobin spray.[76,78]

Wounds caused by the effects of external beam radiation therapy and treated with HBOT[123–130] have offered positive, conclusive outcome data using a 'condition-specific'[8] radiotherapy validated clinical outcome score. These patients generally demonstrate an increase in both their HRQoL and clinical outcome score. This is particularly evident in patients receiving HBOT for recovery from the effects of primary treatment (radiotherapy) of head, neck, bladder or bowel cancer.

There is limited HRQoL data associated with TO.[131] It is advocated that further detailed work should be considered and that endpoints identifying the patient's perspective are needed to show improved quality of life.

Comprehensive reviews from several authors[82,131,132] have reported that careful patient selection is essential in providing the best outcome for the patient. Health professionals are responsible for ensuring the patient is matched to the treatment to provide a positive, synergistic result.

Patient education

Information and education shape a patient's perspective about the treatment they are about to choose or undertake. It is therefore essential that comprehensive, easily understood information and education is offered to the patient[133] before any collaborative health-care decision being made. Sykes and FitzGerald[134] offered the four 'rights' of health literacy; right information, right literacy level,

Table 5 Frequently asked questions

	HBOT (hyperbaric chamber)	Topical oxygen therapy		
		Oxygen-releasing wound dressings	Oxygen diffusion enhancer	Topical oxygen perfusor / chamber
Pain Increase or decrease? Management of pain during treatment	Pain medication can be administered while inside the multi-place chamber.	No evidence	Demonstrate reduction in pain scores	No information available
Recommended therapeutic dose How many treatments do I need? How often do I need them?	Daily treatment sessions Often 2 hours in length 5 days per week (normally Monday—Friday) Number of treatments is dependent on condition. Ranges from 2 or 3 to over 40	Little information regarding generic dosage, length of time and use etc.	Twice per week application to coincide with routine dressing change. Standard container has 30 average wound size applications. Number of treatment depends upon wound healing stage. Takes 5 seconds to apply actual product following wound bed preparation	Topical oxygen chamber: Number of treatments is dependent on condition. Ranges from 2 or 3 to over 40, from 3 times per week up to daily treatment sessions. Up to two hours a treatment Topical oxygen perfusor: treatment 7 days a week for 24 hours
Side effects What I might experience	Visual changes—myopia (short sightedness) can occur after approximately 20 treatments. Vision usually returns to normal over time	No known detrimental effects to the wound bed	No side effects, reactions or allergies to product	No side effects, reactions or allergies to products
Probability of improvement What can I expect with the process of healing	Does not immediately heal the wound HBOT provides highly oxygenated blood and creates a physiologically improved environment for healing	Limited evidence to healing potential. Promoted as supplying unobtrusive oxygen directly to the wound	Positive impact upon slough elimination and exudate reduction Granulox works to increase oxyhaemoglobin to the wound bed cells	Topical oxygen chamber: limited evidence of healing potential Topical oxygen perfusor: provide continuously pure oxygen to wound surface to stimulate wound healing
Changes in routine How does this treatment affect my routine?	It is time consuming, may need to travel to the hyperbaric chamber and daily treatment will most likely take about 2 hours	Device has to be worn close to the body and may thus interrupt patients activities of daily living	No change to patients daily routine. Patients can apply the product at their convenience	Topical oxygen chamber: Yes—may need to travel to the chamber and daily treatment will most likely take about 2 hours Topical oxygen perfusor: has to be worn close to the body, but no change to patients daily routine

Can I stop without disadvantage? To my health, wound etc.	Yes—can cease HBOT or take a break. However break in treatment is discouraged, evidence supports continuity	There are no disadvantages to stopping the product suddenly	There are no disadvantages to stopping the product suddenly	There are no disadvantages to stopping the product suddenly
Complications Is there anything that I should consider that I will need to change in my life so I can have this treatment safely?	Patients with diabetes are likely to experience changes in blood glucose metabolism that will necessitate adjustment in diet and medication supervised by the doctor	Suitability of wearing device depending on location of wound	There are no considerations in regards to treatment safety	There are no considerations in regards to treatment safety
Table elaborated by Carol Baines and Sharon Hunt (Lead Advanced Nurse Practitioner, Independent specialist in wound care, Wellway Medical Group)				

right modality and right time, with 'due respect for any cultural, language and socioeconomic barriers'. O_2 therapy education is based on these essential components and allows the choice to commence O_2 therapy and which type/method of treatment/ O_2 delivery is most suited to their situation to be made in a supported patient focused manner.

All O_2 therapies are challenging to describe by words alone thus the use of multimedia technology has allowed health professionals to improve and transcend this gap.

Before admission to a HBOT service, patients are offered information (in all formats) that details what to expect and how to behave in a hyperbaric chamber. Frequently asked questions such as, 'Who will be responsible for my dressing?' and 'How long is treatment? and 'What type of entertainment can I expect during treatment'? are addressed. There are online virtual tours of hyperbaric facilities while other HBOT services offer 'dry runs' (where patients can sit in a chamber for the experience) and open days to increase public awareness.

Clinical facilities are also engaging with social media and in doing so they offer humanistic patient experiences via contemporary photographs and videos. It is noted that some of the larger hyperbaric services in the US maintain online support groups and peer-to-peer education.

The application of topical O_2 in the home has been documented to be an easy process.[135,136] DVDs, leaflets and peer education has been made available for patients that explain the process, which encourages independence and personal autonomy.

Patient experience

There is little published qualitative research into the 'lived experience' of patients undergoing hyperbaric treatment in a mono-place (single occupancy chamber) or multi-place/patient (several patients being treated at the same time in one chamber) chamber, topical O_2 treatment or O_2 enhancing product (haemoglobin spray).

In research undertaken in old 'deck style' multi-place, cylindrical hyperbaric chambers[137,138] patients reported cold noisy air, feeling uncomfortable sitting, and felt only slightly reassured when they watched 'desensitisation' videos before treatment. Knight[139] wrote of his personal experience that 'treatment is dull' while another study[140] found

that patients felt that their 'life was on hold' while they committed to a daily treatment schedule for 30 treatments. However, these types of chambers are no longer appropriate for use in a clinical medical setting. Hyperbaric chambers are now built to resemble large square rooms, furnished in a familiar 'clinical' style with television monitors and air conditioning. Patients are able to sit or lie comfortably and watch a movie to while away the treatment time. Additionally, the mono-place chamber has added to the hyperbaric suite of options and has certain logistical benefits over multi-place chambers[140] such as fitting treatment time in around work schedules.

Surveys and focus groups conclude that patients' 'lived experience' of hyperbaric therapy in a multi-place chamber is a generally pleasant experience, is person centred,[121,130,140] can be sociable and companionable, and allows/encourages strong peer support situations. However, it was also noted that it can be physically and mentally demanding, time consuming and sometimes burdensome. Katarina et al.[121] presented evidence offered by patients that the continuity of care and consistent clinical message provided by a HBOT team was of great value.

The patient experience of TOT has been explored in a limited context. Gordillo[53] and Orsted[131] provided evidence-based recommendations for practice and comment that the use of this therapy is well adopted by patients.

Several authors[78,135,136,141] have noted a high level of patient acceptance of a haemoglobin treatment, specifically the spray method and have reported on the ease of product use for the patient

Conclusion

This chapter reviews available published data to offer details of the patient's perspective on care with either HBOT, TOT or haemoglobin-enhancing products. The ability to increase O_2 delivery and consequently improve wound healing is a dynamic, evolving field. Despite the paucity of evidence, it seems likely that the patient's perspective will impact on their uptake, experience and the perceived success of O_2 therapy for wound management. This highlights the opportunity and responsibility of the health professional to shape, research, understand and respond to the patient's perspective in order to corroboratively achieve healing.

Recommendations

Large scale, qualitative research is required to focus on specific areas of the patient perspective of oxygen treatment, especially:

- Measurement of patient outcomes associated with O_2 treatment
- HRQoL of patients receiving O_2 treatment
- Advantages of O_2 therapy for the patient from their perspective.
- Exploration and expansion of research into health literacy associated with O_2 treatment. Research to explore the use of HBOT in the treatment of specific skin/wound conditions.

7. Economics

There is some direct evidence on the cost-effectiveness of HBOT in the treatment of acute and chronic wounds.[125,142] A position statement for TOT for chronic wounds by the Undersea and Hyperbaric Medical Society (UHMS) dated 2005 stated that application of TOT should not be recommended before having scientific evidence of its effectiveness.[38] Also, the International Working Group on Diabetic Foot (IWGDF) published in 2015 guidance on the use of interventions to enhance the healing of chronic ulcers of the foot of patients with diabetes giving a strong recommendation, even though based on low-level evidence, that:

'[medical practitioners should] not select agents reported to improve wound healing by altering the biology of the wound, including growth factors, bioengineered skin products and gases, in preference to accepted standards of good quality care'.[143]

There is an increasing amount of evidence for the effectiveness of TOT, at least in specific subpopulations of patients, which is promising due to the relatively low cost of application of TOT.[135,144] In general there is a need for further studies that include economic outcomes in order to make recommendations on the cost-effectiveness of applying HBOT or TOT or both in wound care.

Cost efficiency of individual treatment principles

A limited number of studies have used a double-blind approach to evaluate the efficacy of HBOT in the treatment of DFUs. Gomez-Castillo reported 2003–2004 Australian data that the average cost for wound care and HBOT was AUD14,928 for each amputation prevented, and that HBOT might decrease the overall cost of health care when the costs of amputation and rehabilitation were considered.[145] In Italy the economic indicators for using HBOT in DFUs showed potential saving of €19,000 per patient, which represents about 35% savings.[146] Chuck used 2008 Canadian data on DFU prevalence and HBOT efficacy data to create a computer model that estimated the 12-year cost for patients receiving HBOT was CAD40,695, compared with CAD49,786 for standard care alone.[147] One prospective RCT evaluated the cost of ulcer dressings per visit per patient for one year in both the treatment and control groups and found an average savings of UK£2,960 per patient treated with HBOT.[148]

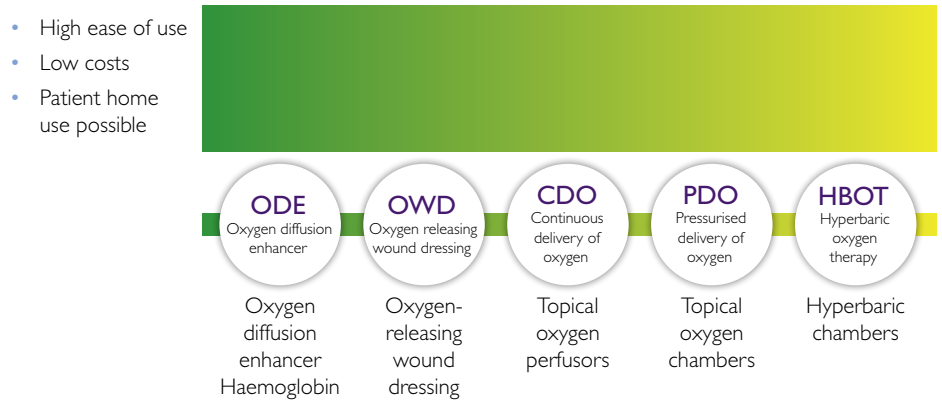
The value of the HBOT for the money spent has been estimated in several countries considering the number needed to treat (NNT).[149] In order to have a homogenous value for money spent, the cost of amputation was standardised for the NHS-UK value.[150] The considered NNT for patients with DFUs is four for up to 35 HBOT sessions and three for more than 35 HBOT sessions.[106,151] In all the Countries evaluated, the HBOT cost is from neutral to likely saving (except Norway and the US due to the high cost of HBOT sessions). However, the cost-effectiveness of HBOT could not be considered as established so long as robust health economic data based on evaluation of large placebo-controlled RCTs evaluating the effect of HBOT as adjunctive treatment in DFUs patients is lacking.[152]

An RCT, which analysed costs in a group treated with O_2-releasing dressings compared with standard of care, failed to show significance. The mean cost per patient treated with the O_2 releasing dressings was £436.33, compared with £525.54 per patient for standard care. Mean cost per ulcer healed at 12 weeks or earlier was £976.54 compared with £1071.29 per patient for standard care only. The cost saving is based on a reduction in the mean number of nurse visits from 14.8 visits for standard care patients to 10.04 visits for patients obtaining the O_2-releasing dressing.[144] UK-based clinical studies have shown that, when added to standard care, haemoglobin spray could save the UK health-care system an average of £2,330 for every patient with a DFU and £1,469 for every chronic wound patient after six months.[135] Thus, there is an increasing clinical evidence that such adjunctive treatment has a positive impact on wound healing and cost reduction.

Where are we today regarding reimbursement in Europe?

The situation is very heterogeneous. In some countries HBOT is paid for by the health system, in other countries it is not. In the US for HBOT to be reimbursed, a facility must ensure the provider supervising the treatment meets Centers for Medicare & Medicaid Services (CMS) requirements. Physicians who supervise HBOT should be certified in UHMS or must have completed a 40-hour, in-person training programme by an approved entity. In addition, if HBOT is performed off-site from a hospital campus or in a physician's office, Advanced Cardiac Life Support training and certification of the supervising physician are required. CMS also requires appropriate direct physician supervision for coverage, meaning that the physician must be present on the premises and immediately available to furnish assistance and direction throughout the performance of the procedure.

Fig 2. General considerations for use of oxygen therapies



This figure does not imply any specific sequential use of different oxygen therapies. Decision on choice of appropriate therapy or concomitant use of different therapies belongs to the physician and depends on clinical status of the patient and the wound as well as availability of the resources.

TOT is not burdened by such requirements and is paid as part of local wound treatment. As they are less expensive than HBOT any prevented amputation should be cost-effective.

This figure does not imply any specific sequential use of different oxygen therapies. Decision on the appropriate choice of therapy or concomitant use of different therapies belongs to the physician and depends on clinical status of the patient and the wound as well as availability of the resources.

Cost-effectiveness

The cost-effectiveness of HBOT and TOT in wound healing is difficult to estimate as it strongly depends on type of payment for both medical procedures and services as well as for general health-related costs (such as rehabilitation, sickness benefits, compensation for disablement etc.). Therefore such analysis is a country-dependent process. However, there are some reports showing that using HBOT or TOT or both as an adjunct for general medical approach might be a cost-effective procedure.

Conclusion

Using HBOT or TOT or both as an adjunct for general medical approach might be cost-effective.

Currently, there is some direct evidence on the cost-effectiveness of HBOT in the treatment of acute and chronic wounds. In DFUs HBOT might decrease the overall cost of health care when the costs of amputation and rehabilitation were considered. Considering the NNT in DFUs, the HBOT value for money spent is from neutral to likely saving for the health system.

In the past, some position statements maintained that the application of TOT should not be recommended before having scientific evidence of its effectiveness but, recently there is increasing evidence on the effectiveness of TOT due to its relatively low cost of application, at least in specific subpopulations of patients. The cost saving of O_2-releasing dressings is especially based on a reduction in the mean number of nurse visits. Furthermore, haemoglobin spray as an adjunct treatment seems to have a positive impact on wound healing and cost reduction.

The reimbursement is very heterogeneous. In some countries HBOT is paid by the health system, in other countries not. TOT is mostly paid as part of local wound treatment and any prevented amputation should be cost-effective.

Recommendations

- In general there is a need for robust health-economic data based on evaluation of large placebo-controlled RCTs in order to make recommendations on the cost-effectiveness of applying HBOT or TOT or both in wound care (GRADE 1)
- As standard of care HBOT should always be used as part of a multidisciplinary treatment plan with ongoing wound care on a regular basis and not as a stand-alone therapy (GRADE 1B)
- It is recommended to provide standard wound care during at least a four-week period before the application of HBOT (GRADE 1C)
- Vascular screening is recommended in order to evaluate if any revascularisation procedure is indicated before HBOT and TOT or both. (GRADE 1 C (HBOT))
- The creation of a European Wound Register to further evaluate the benefit of HBOT and TOT or both in wound care is recommended (GRADE 1 C).

8. Conclusion

Sufficient availability of molecular O_2 is essential for healing of all kind of wounds. O_2 therapies is a general term that includes among other treatments HBOT and TOT. HBOT has been known for many years and is well-established. This paper presented a synopsis of mechanisms of action, clinical evidence and current recommendations of internationally recognised organisations. Due to its relative novelty and the small number of clinical studies of TOT compared with HBOT, the description of several methods classified as TOT were described in more details.

The document provided an overview of treatment options available, as well as an assessment of the best available evidence on their respective results. In addition, it details specific aspects and current discussions regarding the use of O_2 in wound healing, the role of O_2 and hypoxia in the wound healing process, patient perspectives of these treatments, the cost-effectiveness of O_2 therapies as well as discussions of what remains controversial and suggestions for future actions.

The clinical evidence for the efficacy of TOT is not homogeneous and ranges from uncontrolled case reports to RCTs with some limitations. In spite of this adjunct therapies are easy to handle, safe and may be potentially effective modalities for use in modern strategies of wound care in specific subpopulations.

There is evidence that HBOT improves healing by reoxygenation of tissues, exerting an anti-infective effect on both aerobes and anaerobes, decreasing inflammation and oedema, stimulation of angiogenesis and vasculogenesis as well as stem cells in specific subpopulations.

The important question about the concomitant action of TOT with other therapeutic procedures, including HBOT, vascular interventions or skin transplantation, is still unanswered. However, there is an increasing amount of clinical data available on the efficacy of TOT. The patient's perspective seems likely to have an impact on their uptake, experience and the perceived success of O_2 therapy for wound management. Relating to this most TOT procedures can be easily carried out in everyday clinical or home-based practice. Moreover there is some evidence that HBOT and TOT had been used economically in specific clinical settings.

Overall the authors feel that this document helps to clarify the present status in the important treatment modalities dealing with O_2 especially to the patient with non-healing wounds. This information may help the current planning and show the great potential for future treatment strategies.

9. Future perspectives

Oxygen is a pivotal substance in wound healing including infection, and the clinical and scientific interest on its role will improve in the future.

To date, diagnostic tools for measuring local hypoxia have not been adequately used. For further clinical decisions it would therefore be meaningful to use the available measurements regularly, and to improve such techniques. Further studies should demonstrate which treatment modality would be the best for the patient. Yet another point concerns smart dressings, which could incorporate specific sensors and actively modify environmental conditions within the wound.

Thus, targeted patient selection could be performed. This would be a first step towards individualised wound therapy in the near future. Also, there is a distinct need for well-designed prospective and controlled studies to critically evaluate the efficacy and effectiveness of O_2 treatment for the management of non-healing wounds.

In particular with increasing antibiotic resistance the antimicrobial effects of O_2 should be part of future strategies.

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Appendix A

GRADE recommendation explanation

The committee used the GRADE approach (Grades of Recommendation Assessment, Development and Evaluation) system[153] to rate the quality of evidence (confidence in the estimates) and grade the strength of recommendations. This system, adopted by more than 70 other organisations, categorises recommendations as strong GRADE 1 or weak GRADE 2, based on the quality of evidence, the balance be-tween desirable effects and undesirable ones, the values and preferences, and the resources and costs.

GRADE 1 recommendations are meant to identify practices where benefit clearly outweighs risk. These recommendations can be made by clinicians and accepted by patients with a high degree of confidence. GRADE 2 recommendations are made when the benefits and risks are more closely matched and are more dependent on specific clinical scenarios. In general, physician and patient preferences play a more important role in the decision-making process in these latter circumstances.

In GRADE, the level of evidence to support the recommendation is divided into 3 categories: A (high quality), B (moderate quality), and C (low quality). Conclusions based on high-quality evidence are unlikely to change with further investigation; whereas those based on moderate-quality evidence are more likely to be affected by further scrutiny. Those based on low-quality evidence are the least supported by current data and the most likely to be subject to change in the future.

It is important to recognize that a GRADE 1 recommendation can be made based on low-quality (C) evidence by the effect on patient outcome. A full explanation of the GRADE system has been presented to the vascular surgery community.[153,154] A consensus of the recommendations and level of evidence to support it was attained and every recommendation in this guideline represents the unanimous opinion of the task force. Although some recommendations are GRADE 2 with Level 3 data, the task force deemed it appropriate to present these as the unanimous opinion of its members regarding optimal current management. This was done with the understanding that these recommendations could change in the future but that it was unlikely that new data would emerge soon. These guidelines are likely to be a 'living document' that will be modified as techniques are further refined, technology develops, medical therapy improves, and new data emerge. The Committee monitored the literature for new evidence emerging after the search of the 5 commissioned systematic reviews and the group periodically updated guidelines as new data became available.

Table 6 GRADE approach to treatment recommendations

Recommendation	Benefit vs risk	Quality of evidence	Comment
1A	Clear	High: Consistent results from RCTs or observational studies with large effects	Strong recommendation, generaliseable
1B	Clear	Moderate: RCTs with limitations and very strong observational studies	Strong recommendation; May change with further research
1C	Clear	Low: Observational studies Very Low: Case series, descriptive re-ports, expert opinion	Intermediate recommendation; Likely to change with further re-search
2A	Balanced or Unclear	High: Consistent results from RCTs or observational studies with large effects	Intermediate recommendation: May vary with patient values
2B	Balanced or Unclear	Moderate: RCTs with limitations and very strong observational studies	Weak recommendation; May vary with patient values
2C	Balanced or Unclear	Low: Observational studies Very Low: Case series, descriptive re-ports, expert opinion	Weak recommendation; Alternative treatments may be equally valid
Adapted from Guyatt G, Schunemann HJ, Cook D, Jaeschke R, and Pauker S. Applying the grades of recommendation for antithrombotic and thrombolytic therapy. Chest 2004; 126; 179S-187S.			

Evidence-based practice standards for the use of topical pressurised oxygen therapy

Heather L Orsted, Randy Poulson, and the Advisory Group (Joseph Baum, Dawn Christensen, Marc Despatis, Kyle Goettl, David Haligowski, Chester Ho, Keith Louis, Deirdre O'Sullivan-Drombolis, Valerie Winberg and Kevin Y Woo)

Orsted HL, Poulson R, and the Advisory Group. Evidence-based practice standards for the use of topical pressurised oxygen therapy. Int Wound J 2012; doi: 10.1111/j.1742-481X.2012.00956.x

ABSTRACT

Whenever a new therapy enters the wound care arena it is mandatory to deliver the best evidence to clinicians, healthcare administrators and policy makers to support integration of the technology into clinical practice. While this can often be problematic when novel therapies lack a large body of supporting evidence, methods that incorporate the use of expert opinion do exist to evaluate existing evidence and create consensus statements that can help guide decisions. Topical pressurised oxygen therapy is a method of delivering pressurised and humidified oxygen directly to the wound bed to support the healing of chronic and hypoxic wounds. This article will present the process by which the evidence was identified and evaluated as well as present standards based on the evidence related to topical pressurised oxygen therapy. We will show, through the use of the evidence, how this therapy can be a non invasive safe approach for wound management for selected patients in all clinical care settings.

Key words: Evidence-based • Oxygen therapy • Pressurised

INTRODUCTION

Canada's aging population will soon become 'an $850 billion ticking time bomb' (1) and the management of wounds will take-up a large part of these rising healthcare costs.

An Ontario-wide study has shown that wound care accounts for up to 50% of home care services provided at any given time. The project estimates that 31 000 people are admitted to home care for wound care each year, with an annual cost of $108·7 million in services with respect to wounds, excluding the cost of supplies and equipment (2).

In 2010, Ontario, a province in Canada, introduced Bill 46, which calls for 'excellent care for all' through legislation that puts the patient first. The foundation for this is a high-quality healthcare system that is 'accessible, appropriate, effective, efficient, equitable, integrated, patient-centred, population health focused and safe' (3).

Reconciling the realities of a need for 'excellent care for all' and increasing healthcare costs is an issue facing every healthcare jurisdiction in Canada, and elsewhere around the world.

Key Points

- reconciling the realities of a need for 'excellent care for all' and increasing healthcare costs is an issue facing every healthcare jurisdiction in Canada, and elsewhere around the world

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 doi: 10.1111/j.1742-481X.2012.00956.x

Key Points

- topical pressurised oxygen therapy* is a therapeutic modality that delivers humidified, pressurised oxygen directly to a specific body part to achieve tissue penetration and increased oxygen levels to the open ischaemic wound

How do decision-makers decide on the most cost-effective choices while continuing to provide excellent care, specifically in relation to wound care?

Innovative technologies for addressing wounds can sometimes answer the need, but must be carefully reviewed by wound care clinicians, researchers and decision-makers to determine their level of evidence before implementation into practice. If warranted, they can be part of the solution by reducing wound healing times and decreasing wound recurrence rates – thereby meeting the standards identified by Bill 46.

New technologies rarely come with an abundance of evidence to back them. So how do decision-makers evaluate innovations? What can the evidence of related but not identical technologies offer to the questions surrounding the new technology? How are gaps in the evidence that may exist, be identified and filled? How can decision-makers and practitioners critically evaluate anecdotal evidence presented by eager proponents of the new technology? How can the manufacturers/distributors of the technology represent their product fairly, i.e. in the best light while conforming to best evidence standards?

These were exactly the questions that needed to be addressed regarding topical pressurised oxygen therapy, a relatively new wound treatment modality that has abundant anecdotal evidence that parallels other technologies but lacks a large body of specific evidence to support its use.

To answer these and other important questions, a process was implemented that addressed the following concerns:

1. Bias: It is essential to the integrity of the process that an individual with no stake in the outcome facilitates all activities regarding the evaluation of the evidence.
2. Method: To ensure that the outcomes have value to the field, the process used must be validated, transparent and well understood by all participants.
3. Experience: An interprofessional group of individuals with long experience in the area where the technology is used is ideal to provide a range of viewpoints and expertise during the process of evaluating the evidence.
4. Experience with the specific technology: The hands-on experience of practitioners in the field can provide another essential viewpoint, particularly when there is a lack of research in the literature.
5. Range of evidence: Finding different levels and types of evidence is important when there is not a lot of evidence to evaluate. A variety of evidence often provides insight into strengths and weaknesses of the total body of evidence and can more easily lead to identification of where the gaps are.
6. Realistic presentation of findings: Once a process is in place to evaluate the evidence, the reporting of that evaluation must be presented in such a way that:

- appropriate follow-up questions can be asked,
- useful future research can build on the information reported,
- decision-makers can be confident in any decisions they make to accept, reject or defer the use of the technology.

WHAT IS TOPICAL PRESSURISED OXYGEN THERAPY?

Topical pressurised oxygen therapy* is a therapeutic modality that delivers humidified, pressurised oxygen directly to a specific body part to achieve tissue penetration and increased oxygen levels to the open ischaemic wound. Although hyperbaric therapy has been used for a century, topical pressurised oxygen therapy is relatively new, having been developed in 1969 by Dr Fischer, an Austrian engineer, physician and faculty member of the Institute of Rehabilitation Medicine at New York University (4).

Studies have shown that topical pressurised oxygen therapy raises tissue O_2 levels to a depth of 2 mm within the wound bed, stimulates new blood vessel formation, supports synthesis and maturation of collagen deposition, leading to increased tensile strength and decreased recurrence of the wound. Increased oxygen levels at the wound site have shown to lead to the timely closure of wounds.

*Topical pressurized oxygen therapy (TPOT) is approved by the Therapeutic Products Directorate as a Class 2 Medical Product through Health Canada, Health Products and Food Branch.

According to the distributor, topical pressurised oxygen therapy is currently available in the US under national contract for all Veterans Affairs Medical Centers to be used in the hospitals or in the home care setting. It is currently approved in five states for medical assistance recipients, with three more states in the approval mode. There are hospitals in Ohio that use them in the inpatient setting. Currently in Canada, topical pressurised oxygen therapy has been used in British Columbia, Ontario and Quebec.

HOW DOES TOPICAL PRESSURISED OXYGEN THERAPY DIFFER FROM OTHER FORMS OF OXYGEN THERAPY?

Systemic hyperbaric oxygen therapy (HBOT) is a treatment modality in which the patient breathes 100% oxygen at a pressure greater than one atmosphere: the pressure of air at sea level. This therapy occurs while the patient is entirely enclosed in a stationary pressure chamber. This therapy increases the plasma oxygen levels and is systemic, therefore dependent on adequate blood-flow to the wound. As HBOT is systemic and raises the pO_2, there is a risk of complications such as seizures, damage to the tympanic membrane of the ear (barotraumas) and damage to the retinal nerve (retinopathy). If patients have diabetes their glucose levels could also be affected by an increased pO_2.

Topical pressurised oxygen therapy is also considered hyperbaric in that it also delivers 100% oxygen at a pressure greater than one atmosphere. However, it is a non invasive, portable therapy that uses a reusable acrylic chamber, vinyl extremity boot or vinyl multipurpose bag to deliver humidified pressurised oxygen directly to the wound bed. This method of delivery achieves tissue penetration and increased oxygen levels in the open wound without risk of systemic oxygen toxicity. Topical pressurised oxygen therapy is not dependent on systemic circulation reaching the wound bed.

Topical continuous oxygen therapy is the delivery of non pressurised, non humidified oxygen to the open wound via a cannula placed over the wound with a dressing topper.

USING NEW TECHNOLOGIES

All new technologies must be approved for use by the Food and Drug Administration (FDA in the United States) and by the Therapeutic Products Directorate, Medical Product through Health Canada, Health Products and Food Branch (in Canada). Products must be approved for safety issues such as electrical configuration, electromechanical interference (EMI), pressure testing and also to verify that each product does what the manufacturer says it does. Case studies and the science supporting the product need to be approved for the specific use indicated. Upon submission of an application for approval, the approval organisation reviews the diagnosis such as diabetic foot ulcer, venous stasis ulcer, pressure ulcer and examines how and why the device works. Topical pressurised oxygen therapy* is a licensed microportable version of a hyperbaric chamber and Health Canada granted licensing based on its safety and treatment effectiveness of chronic problem wounds (including diabetic foot ulcers), which is one of the 13 recognised the Undersea Hyperbaric Medical Society (UHMS) indications for hyperbaric treatment that we use to license hyperbaric chambers.

Although topical pressurised oxygen therapy is approved for use and studies have showed its effectiveness, there are still many questions that clinicians need to ask in order to maintain a high standard of care. This is true for any new – or even existing – technology:

1. Is this therapy appropriate for my patient and his/her wound at this point in time, considering indications, contraindications, precautions and warnings?
2. Has the physician/advanced practice clinician ordered the therapy with appropriate duration and frequency?
3. Is the type of device appropriate for the wound type?
4. Are the members of the wound care team properly trained in the use of this therapy?
5. Has the patient and his/her caregivers been trained in how to apply and/or monitor the device and what to do and who to contact in case of problems or emergencies?
6. Are the appropriate wound dressing materials being used?

Key Points

- although topical pressurised oxygen therapy is approved for use and studies have showed its effectiveness, there are still many questions that clinicians need to ask in order to maintain a high standard of care
- in an effort to address these questions and others, an independent, inter professional advisory group (AG) was assembled to aggregate and weigh the evidence, set a standard for the delivery of topical pressurised oxygen therapy and determine where there were gaps in the evidence

Key Points

- the process chosen to develop the set of standards was the Delphi method
- the Delphi method has been linked with the term 'collective intelligence' used to support the development of a knowledge base by structuring a group communication process to facilitate consensus building and group problem-solving
- the product from this approach can lead to the dissemination and implementation of findings such as the publication of consensus statements that can guide health policy, clinical practice and research

7. Is there a standard for wound re-evaluation to determine the therapy's effectiveness and when it can be discontinued?
8. Have appropriate arrangements been made for use of this therapy across the continuum of care (acute care, long-term care, home care, outpatient)? Adapted from reference 5.

In an effort to address these questions and others, an independent, interprofessional advisory group (AG) was assembled to aggregate and weigh the evidence, set a standard for the delivery of topical pressurised oxygen therapy and determine where there were gaps in the evidence.†

METHODOLOGY – THE DELPHI METHOD

The process chosen to develop the set of standards was the Delphi method. The Delphi method has been linked with the term 'collective intelligence' used to support the development of a knowledge base by structuring a group communication process to facilitate consensus building and group problem-solving. The product from this approach can lead to the dissemination and implementation of findings such as the publication of consensus statements that can guide health policy, clinical practice and research (6).

The advisory group

A Canadian interprofessional AG was selected to participate in the Delphi process. This group was chosen based on discipline and geographic location. Each individual also needed to meet four 'expertise' requirements: (i) knowledge and experience with wound care and/or topical pressurised oxygen therapy, (ii) capacity and willingness to participate, (iii) sufficient time to participate and (iv) effective communication skills.

The interprofessional members of the AG are

1. **Joseph Baum:** MD, FRCS(C), Department of Surgery, Division of Plastic Surgery, Etobicoke General Hospital, Ontario. He is a plastic surgeon with over 30 years experience treating complex surgical wounds. He has a special interest in wound care, introducing clinical use of negative pressure wound therapy (NPWT) to Canada, and is physician leader of a committee organising wound care at Etobicoke General Hospital. He has used topical pressurised oxygen therapy on wounds therapeutically on both inpatient and outpatient bases.
2. **Dawn Christensen:** BScN, RN, MHSc(N), CETN(C), Clinical Nurse Specialist/Advanced Practice Nurse, KDS Professional Consulting, Ontario. She has been an enterostomal therapy nurse (with expertise in advanced wound care) since 1989 and currently consults on wound care at 30 long-term care facilities and two community acute care hospitals in the Ottawa area. She is currently a member of and was a board member for the Canadian Association for Enterostomal Therapy and is a member of the Canadian Association of Wound Care.
3. **Marc Despatis:** BSc, MSc, RVT, MD FRCS, Vascular Surgery, Centre Hospitalier Universitaire de Sherbrooke, Quebec. He has many years of experience in wound care. He has been part of specialised clinics (diabetic foot ulcer and venous leg ulcer) working in multidisciplinary care in a university hospital. He has been involved with the Canadian Association of Wound Care over the last 10 years. He has no clinical experience with topical pressurised oxygen therapy.
4. **Kyle Goettl:** RN, BScN, MEd, IIWCC, Nurse Clinician, Amputee Rehabilitation, Parkwood Hospital, London, Ontario. He is a member of the Canadian Association of Wound Care, the Canadian Diabetes Association and is a graduate of the International Interprofessional Wound Care Course through the University of Toronto. He is also an associate scientist at the Lawson Health Research Institute and sits on the Medical Advisory Council for the Amputee Coalition of Canada. He is a member of the Chronic Wound and Skin Healthcare team at Parkwood Hospital and has worked in many specialty areas and as a research study

†TSS, the Canadian distributors of topical pressurized oxygen therapy, engaged services of eQuadra Solutions Inc. (London, ON) to assemble an advisory group of 10 experts. Through the use of a modified Delphi method, eQuadra facilitated a process for arriving at consensus statements and identifying gaps in the evidence.

nurse on various projects. He has been involved in many initiatives to advance best practice wound care and prevention of wounds for a wide variety of patient populations. He has had direct involvement in the selection and trialing of topical pressurised oxygen therapy on several inpatients at Parkwood hospital.

5. **David Haligowski:** BSc, MD, Family physician, Lecturer and Sessional instructor, University of Manitoba, member of the Uniting Primary Care and Oncology and Medical Director of Middlechurch Home of Winnipeg and River East Personal Care Home, Manitoba. He is a former director of the Canadian Association of Wound Care.
6. **Chester Ho:** MD, Physiatrist, Associate Professor and Head, Division of Physical Medicine and Rehabilitation, Department of Clinical Neurosciences, University of Calgary, Alberta. He has over 10 years of advanced wound care experience and founded the interdisciplinary skin care team and was the cochair of Skin Care Committee at Louis Stokes Cleveland Department of Veterans Affairs Medical Center. He has presented nationally and internationally on pressure ulcer management and also has an active research program on pressure ulcer issues, with research funding from national agencies and multiple peer-reviewed publications on this topic. He has written many chapters in major Physical Medicine and Rehabilitation textbooks on the topic of pressure ulcers. He has used topical pressurised oxygen therapy clinically in his previous practice in Cleveland and in his current practice in Calgary with spinal cord injury patients with non healing, stage IV pressure ulcers.
7. **Keith Louis:** MD, Fellowship in general and vascular surgery, in practice since 1985 with a special interest in diabetic wounds, Ontario. He is currently involved in the wound care clinic at Brampton Civic Hospital sharing coverage with two Infectious Disease specialists. He is frequently consulted on diabetic wounds that are seen in-hospital. He is also on the Canadian board of advisory surgeons for NPWT therapy and its related products. He has been involved in approximately six cases using topical pressurised oxygen therapy.
8. **Deirdre O'Sullivan-Drombolis:** BSc PT, MClSc (Wound Healing), Physical Therapist and Wound Care Team Lead, Riverside Health Care Facilities, Fort Frances, Ontario. She is the wound care team lead and resource for Riverside Health Care Facilities in Fort Frances, Ontario. Her role involves implementing best practices in wound care through the development of policies and procedures, education as well as clinical practice. She is also an adjunct faculty for the University of Western Ontario Clinical Masters in Wound Healing Program and chairs the Northwestern Ontario Wound Community of Practice.
9. **Valerie Winberg:** RN(EC), BScN, MN, NP-PHC, ENC(c), IIWCC, Emergency department, Chatham-Kent Health Alliance, Project lead for Twin Bridges NP-Led Clinic, Sarnia, Ontario. She has been a nursing professional for 25 years with extensive experience in all sectors of the healthcare environment, working many years in the emergency department first as a RN then as an NP, practising in primary care in the community and long-term care, with the last 10 as a primary healthcare nurse practitioner. She was a founding member and an executive for the Ontario Woundcare Interest Group, an interdisciplinary political action group. She participates in wound consultations and wound care education for groups and as an international speaker. She has had experience with topical pressurised oxygen therapy with a variety of patients including First Nations peoples and with DM, venous and lower limb ulcers.
10. **Kevin Woo:** RN, MSc, PhD(c), ACNP, GNC(C), Assistant Professor, School of Nursing Queen's University, Kingston, Ontario. In addition to being on faculty for wound care programs, Dr Woo is an advanced wound care consultant, advisory board member for multiple wound care companies, and international speaker. He has topical pressurised oxygen therapy experience, including both trials and regular/occasional use.

The objective was to bring this group of experts together to create a document that would support efficient and effective clinical decision-making relating to topical pressurised oxygen therapy. The following criteria were followed to represent the characteristics of the Delphi method:

1. Anonymity of Delphi participants: allowed the participants to freely express their opinions without undue pressures to conform to others in the group. Decisions were evaluated on their merit, rather than who had proposed the idea.
2. Iteration: allowed the participants to refine their views in light of the progress of the group's work from round to round.
3. Controlled feedback: informed the participants of the other participant's perspectives, and provided the opportunity for Delphi participants to clarify or change their views.
4. Statistical aggregation of group response: allowed for a quantitative analysis and interpretation of data.

Delphi method

The following steps (Figure 1) were required to complete this process:

Step 1: Attended a conference call to introduce the AG members and discuss the process of the project.

Step 2: The AG members read the resource material provided to become familiar with the evidence supporting topical pressurised oxygen therapy technology.

Step 3: The AG members critically reviewed the draft Standards for Use of Topical Pressurised Oxygen Therapy document and appraised each statement, based on their experience/expert opinion and the supporting documents, by checking the appropriate responses: Agree, Somewhat agree or Disagree.

Step 4: If 'Somewhat agree' or 'Disagree' was checked, a comment was required stating why there was no agreement and identifying a recommendation to correct or improve the statement.

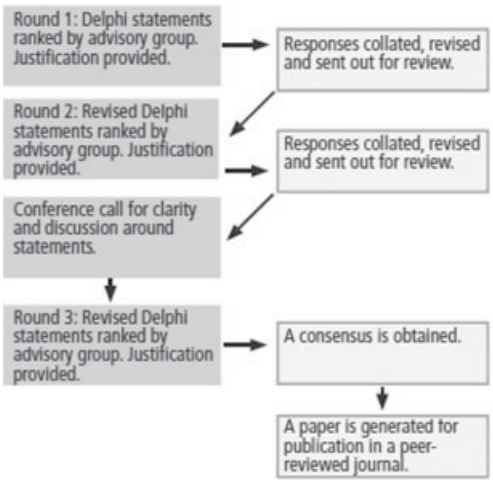

Figure 1. The Delphi method.

Step 5: The AG members returned the draft Standards for Use of Topical Pressurised Oxygen Therapy document for collation.

Step 6: Once all the reviews were received and collated a new document was created by the consultants based on the responses and sent for an additional round of reviews. Standards that did not achieve 80% endorsement were revised along with justification.

Step 7: Continued process (Steps 3 through 6) until consensus on the statements was obtained.

Step 8: A second conference call occurred for further discussion for clarification towards consensus building.

Step 9: Developed a consensus paper for peer-reviewed publication.

Step 10: Set a revision plan for the document (recommended for 3–5 years).

THE RESOURCE MATERIAL

In January 2011, a literature search of PubMed was conducted by the manufacturer (AOTI Inc., West Galway, Ireland) to determine the level of evidence surrounding topical pressurised oxygen therapy. Twenty-seven articles were identified using the following terms: oxygen therapy and wound healing. These articles were reviewed by the consultants from both the distributor and from eQuadra with six articles selected as being current and specific to topical pressurised oxygen

Table 1 Advisory group resource material

Resource material	Description
Topical wound oxygen therapy in the treatment of severe diabetic foot ulcers: a prospective controlled study: Blackman *et al*. (7)	Prospective controlled study
Improving accuracy of wound measurement in clinical practice: Flanagan (8)	Review of the literature
Dermal excisional wound healing in pigs following treatment with topically applied pure oxygen: Fries *et al*. (9)	Experimental study using a pre-clinical model
Medical Director for AOTI: Frye (10)	Manufacturer's recommendations
Evidence-based recommendations for the use of topical oxygen therapy in the treatment of lower extremity wounds: Gordillo *et al*. (11)	Summary of experimental, pre-clinical and clinical findings
Topical oxygen therapy induces vascular endothelial growth factor expression and improves closure of clinically presented chronic wounds: Gordillo *et al*. (12)	Non randomised controlled study
New Therapeutic Angiogenesis Biomarkers for Chronic Diabetic Foot Ulcers Treated with Transdermal Hyperoxia/Topical Wound Oxygen (TWO2): Scott (13)	Experimental study
Best Practice Recommendations for preparing the wound bed: Update 2006: Sibbald *et al*. (14)	Summary of recommendations
Does topical wound oxygen (TWO_2) offer an improved outcome over conventional compression dressings (CCD) in the management of refractory venous ulcers (RVU)? A Parallel Observational Study: Tawfick and Sultan (15)	Parallel group observational comparative study

therapy as well as having identified outcomes. Additionally, the manufacturer's website was considered as a resource because it contained recommendations for product use. Two other articles were identified to support a best practice approach to wound management as well as a standard for wound assessment. These nine resources were then used by the AG as the basis for their evaluations of the Standard statements to support the appropriate use of topical pressurised oxygen therapy (Table 1).

STANDARD STATEMENTS

Standard statements were developed from the resource material that best describes the use and usefulness of topical pressurised oxygen therapy and were agreed upon by the AG. The strength of the evidence for each standard was based on the Registered Nurses Association of Ontario (RNAO) Interpretation of the Evidence (Table 2).

The standard statements and related discussion were placed into the following categories:

1. Product description
2. Patient selection
3. Patient preparation
4. Application principles
5. Evaluating therapy
6. Expected outcomes
7. Resources
8. Safety and precautions

The AG, using the Delphi method, finalised the statements and weighted the level of evidence as indicated in the Quick Reference Guide (Table 3).

DISCUSSION OF THE EVIDENCE

Product description

Topical pressurised oxygen therapy is an adjunctive modality/device designed to support wound healing, Level IIa

Discussion: It is well established that oxygen is vital for wound healing through the synthesis of collagen, enhancement of fibroblasts, angiogenesis and leukocyte function. Oxygen also has key functions in energy metabolism and in the inhibition of microbial growth. Oxygen and reactive oxygen species are required and involved in all stages of wound healing: modulating cell migration, adhesion, proliferation, neovascularisation, remodelling and apoptosis (7,9,12,15).

In acute and chronic wounds, a state of hypoxia frequently occurs during the inflammatory phase of wound healing and helps to 'kick-start' angiogenesis; however, increased O_2 is necessary for continued wound healing (12) (Figure 2). Tissue hypoxia caused by disrupted or compromised vasculature can be a key factor that limits wound healing (11). This hypoxic state can occur because of capillary congestion, oedema, peripheral vascular disease (PVD) or peripheral arterial disease (PAD), where the wound does not get an

Table 2 Interpretation of the evidence

Level	Description
Ia	Evidence obtained from meta-analysis or systematic review of randomised controlled trials.
Ib	Evidence obtained from at least one randomised controlled trial.
IIa	Evidence obtained from at least one well-designed controlled study without randomisation.
IIb	Evidence obtained from at least one other type of well-designed quasi-experimental study, without randomisation.
III	Evidence obtained from well-designed non experimental descriptive studies, such as comparative studies, correlation studies and case studies.
IV	Evidence obtained from expert committee reports or opinions and/or clinical experiences from respected authorities.

Table 3 Topical pressurised oxygen therapy: quick reference guide

Category	Statement	Strength
Product description	Topical pressurised oxygen therapy is an adjunctive modality/device designed to support wound healing.	Level IIa
	Topical pressurised oxygen therapy delivers humidified oxygen to the wound bed at cyclical pressures above atmospheric pressure.	Level IIa
	Topical pressurised oxygen therapy delivers oxygen into the wound bed, penetrating into the tissue approximately 2 mm deep.	Level IIb
Patient selection	Topical pressurised oxygen therapy is indicated for the treatment of chronic wounds such as diabetic/neuropathic foot ulcers, venous stasis ulcers and pressure ulcers.	Level IIa
	Topical pressurised oxygen therapy is contraindicated if the patient has an untreated acute DVT or acute thrombophlebitis.	Level IV
Patient preparation	The presence of necrotic tissue must be minimised in the wound bed prior to the initiation of therapy.	Level III
	The cause(s) of trauma and cofactors that may interfere with healing of the wound must be removed prior to the initiation of therapy.	Level IV
	Client and caregiver concerns must be addressed prior to the initiation of therapy.	Level IV
	Topical dressings post-therapy must meet the needs of the wound in terms of debridement and bacterial and moisture balance.	Level IV
	Any dressings or preparations that create an oxygen-impermeable barrier, such as any petrolatum-based product or occlusive dressing, cannot be used in conjunction with topical pressurised oxygen therapy.	Level IV
Application principles	The frequency and duration of therapy is dependent on wound aetiology, wound response and patient tolerance.	Level IV
Evaluating therapy	Patients being treated with topical pressurised oxygen therapy require assessment using standardised instruments and documentation on a regular basis according to agency healthcare setting practice and policy.	Level III
	If wound closure is the goal and the wound is not reduced by 20–40% after 2–4 weeks of therapy despite efforts to address the underlying causes and cofactors, therapy should be discontinued and alternate methods sought.	Level IV
Expected outcomes	Increased wound oxygenation, through the application of topical pressurised oxygen, results in increased collagen deposition and tensile strength.	Level IIa
	Topically applied pressurised oxygen increases angiogenesis-related growth factor expression in wound fluids from chronic diabetic foot ulcers that may be consistent with revascularisation and renewed healing.	Level IIa
	A lower recurrence rate may be expected in venous leg ulcers and diabetic foot ulcers following topical pressurised oxygen therapy.	Level III
	Topical pressurised oxygen therapy may reduce wound-related pain in venous leg ulcers.	Level III
Resources	Education needs to be provided to patients, caregivers and healthcare providers regarding the purpose and process of using topical pressurised oxygen therapy.	Level IV
	Preliminary studies have shown that topical pressurised oxygen therapy has the potential for cost savings.	Level IV
Safety and precautions	Protocols for oxygen safety must be followed when therapy is in use.	Level IV

DVT, deep venous thrombosis.

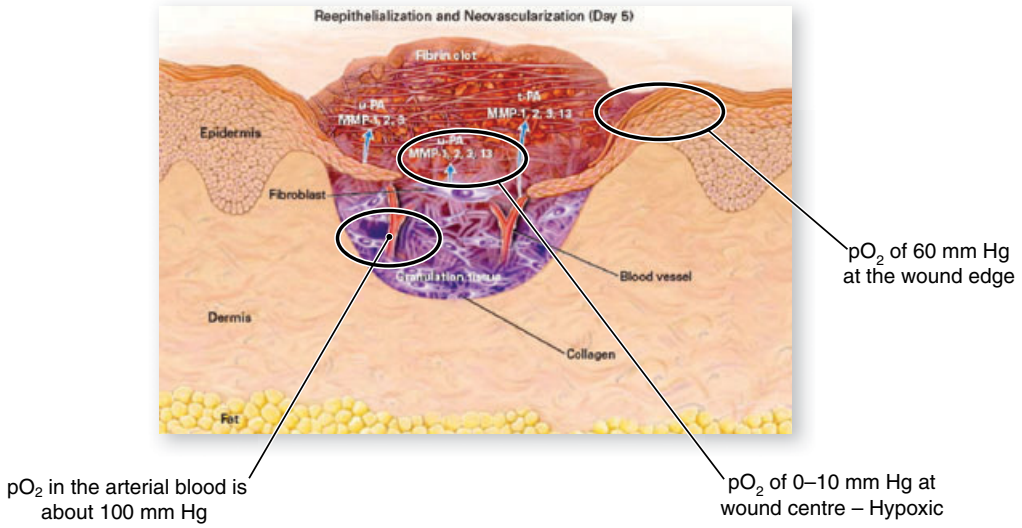

Figure 2. Oxygenation and trauma. Reprinted with permission from reference 10.

adequate supply of oxygenated blood. Studies show that tissues must have a pO_2 of at least 40 mm Hg in order to promote the production of FEGF, vascular endothelial growth factor (VEGF), collagen and most importantly restore angiogenesis and neovascularisation (12).

Topical pressurised oxygen therapy reduces hypoxia, promoting increases in FEGF for collagen formation and VEGF promoting neoangiogenesis (13). Topical pressurised oxygen therapy can generate a sustained increase in wound pO_2, supporting angiogenesis; and in chronic human wounds it can induce a progressively increasing and sustained elevation of VEGF expression (11).

Topical pressurised oxygen therapy delivers humidified oxygen to the wound bed at cyclical pressures above atmospheric pressure, Level IIa

Discussion: Topical pressurised oxygen therapy is a form of hyperbaric medicine in that it uses oxygen at a higher level than atmospheric pressure. The therapy consists of delivering pressurised, humidified 100% oxygen from 1·0 atmosphere absolute (ATA) to 1·03 atmospheres (ATA) topically to the wound bed and periwound skin. The atmospheric pressure increases in a sine-wave amplitude (a smooth repetitive cycle) from baseline to plateau and then back to base line (7,11,12,15).

Topical pressurised oxygen therapy delivers oxygen into the wound bed, penetrating into the tissue approximately 2 mm deep, Level IIb

Discussion: Topical oxygen therapy increases the tissue pO_2 of superficial wound tissue in pigs (9). Using a special probe designed to measure superficial pO_2 at 2 mm depth at the centre of the wound bed, Fries *et al*. saw an increase of pO_2 from less than 10 mm Hg to 40 mm Hg in as little as 4 minutes. Fries *et al*. showed by histology that wounds treated with oxygen that penetrated into the tissues showed signs of improved angiogenesis and tissue oxygenation in pigs (Figure 3).

Patient selection

Topical pressurised oxygen therapy is indicated for the treatment of chronic wounds such as diabetic/neuropathic foot ulcers, venous stasis ulcers and pressure ulcers, Level IIa

Discussion: Topical pressurised oxygen therapy has showed effectiveness as an adjunctive therapy to best practice for the management of acute and chronic diabetic/neuropathic foot ulcers, venous stasis ulcers, some mixed ulcers and pressure ulcers. It can also be an adjunctive therapy for treating wounds where hypoxia, oedema and increased bioburden are suspected to be key factors interfering with wound healing. In two studies with chronic wounds, topical pressurised oxygen therapy has shown to demonstrate no adverse responses (7,11,12,15).

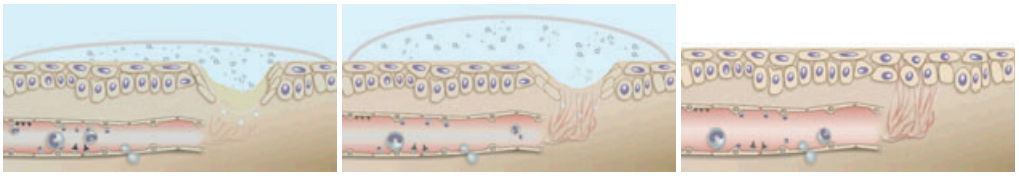

Figure 3. Oxygen penetrates to the cellular level supporting angiogenesis and enhancing collagen formation. Reprinted with permission from reference 10.

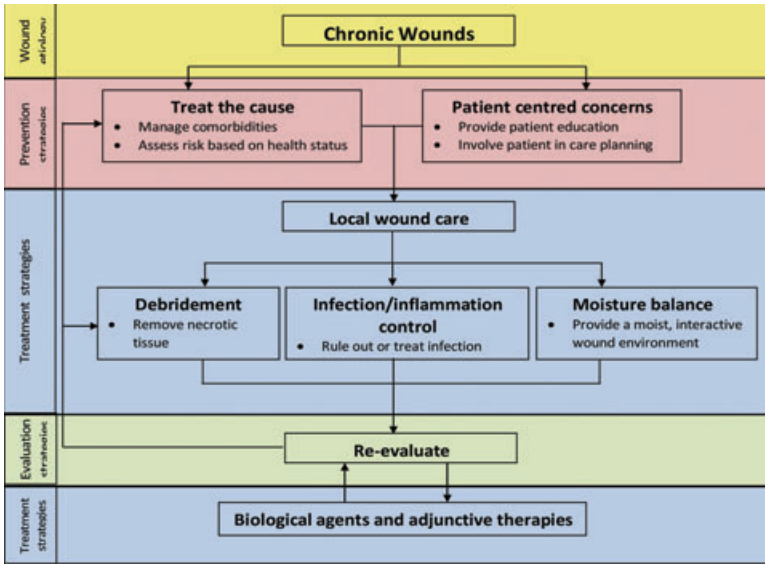

Figure 4. Wound bed preparation paradigm. Adapted with permission from reference 14.

Topical pressurised oxygen therapy is contraindicated if the patient has an untreated acute deep venous thrombosis or untreated acute thrombophlebitis, Level IV

Discussion: In the instance of acute untreated deep venous thrombosis (DVT) or thrombophlebitis, topical pressurised oxygen therapy is contraindicated. The cyclical positive pressure that is delivered by the hard chamber or extremity system may increase the risk that a clot may be dislodged and moved through the circulatory system, possibly promoting stroke, myocardial infarction or pulmonary emboli and risk of sudden death (10).

Patient preparation

The presence of necrotic tissue must be minimised in the wound bed prior to the initiation of therapy, Level III

Discussion: Wounds should have at least 50% viable tissue exposed to allow for adequate oxygen to enter the tissues – therefore, it is imperative to remove as much eschar and slough from the wound bed as safely possible. Wounds can be debrided through surgical, sharp, autolytic, enzymatic, mechanical or larval methods. Topical pressurised oxygen therapy can then deliver pressurised oxygen directly to the surface of the wound, allowing oxygen penetration to achieve its maximum benefit (7,11,12,14,15).

The cause(s) of trauma and cofactors that may interfere with healing of the wound must be removed prior to the initiation of therapy, Level IV

Discussion: The patient must be approached as a whole person. The clinician(s) need to address all the factors and cofactors that could interfere with healing before focus turns to the wound. The wound bed preparation model (Figure 4) promotes wound management through assessment, diagnosis and appropriate treatment of the cause, attention to patient-centred concerns, and only then addresses local wound care. It is important to address factors that may interfere with wound healing through steps such as providing pressure-relieving surfaces to reduce pressure and trauma, proper

offloading to reduce trauma to diabetic foot ulcers and controlling oedema in the presence of venous leg ulcers. There are many cofactors, such as nutrition and hydration that should also be considered. Once these have been addressed, topical pressurised oxygen therapy can be adjunctive with the primary treatment strategies (11,14).

Client and caregiver concerns must be addressed prior to the initiation of therapy, Level IV

Discussion: The interdisciplinary team needs to work closely with patients, caregivers and their families to address the complex lifestyle, self-care and multiple treatment demands of patients who have chronic wounds.

Patient concern is a key component of the wound bed preparation model (Figure 4) and supports patient adherence to therapy (14). Patient and caregiver concerns such as pain management, dressing removal and reapplication, signs and symptoms of infection, equipment usage – including proper application, troubleshooting, cleaning and maintenance – should all be considered and addressed with the patient and their caregivers (10).

Select a topical dressing post-therapy that meets the needs of the wound in terms of debridement and bacterial and moisture balance, Level IV

Discussion: Clinicians should base dressing selection on the patient history and assessment, the cause of the wound, and the evaluation of the wound bed and periwound skin. The dressing should address the needs of the wound with a focus on its ability to support debridement, bacterial and moisture balance (Figure 3). Because the dressing needs to be removed and reapplied once or twice a day the dressing should not cause trauma with frequent removal (14).

Any dressings or preparations that create an oxygen-impermeable barrier, such as any petrolatum-based product or occlusive dressing, cannot be used in conjunction with topical pressurised oxygen therapy, Level IV

Discussion: Many wound care products have components that will prevent or restrict oxygen from penetrating the wound bed. Petrolatum is a semi-solid mineral oil product that is often used in wound dressings and can create an occlusive wound covering that can interfere with topical oxygen delivery. Occlusive barriers, film dressings and any products that may restrict oxygen access to the wound bed should also be avoided during therapy (9–11).

Table 4 Recommended protocols for topical pressurised oxygen therapy.

Ulcer type	Diabetic foot ulcers	Venous leg ulcers	Pressure ulcers
Frequency	OD or BID	BID	OD or BID
Duration	120 minutes	180 minutes	120–180 minutes
Device	Extremity system	Extremity system	Multipurpose bags

OD, once a day; BID, twice a day.

Application principles

The frequency and duration of therapy is dependent on wound aetiology, wound response and patient tolerance, Level IV

Discussion: The manufacturer has recommended protocols for topical pressurised oxygen therapy (Table 4) based on the hyperbaric protocols identified by the UHMS, to determine the frequency and duration of the therapy. However, these may need to be modified based on studies, clinician experience, wound aetiology and patient tolerance (10,11,15,16).

For burns and post-surgical wounds: frequency, duration and devices will be determined based on the location of the wound and orders.

Evaluating treatment

Patients being treated with topical pressurised oxygen therapy require assessment using standardised instruments and documentation on a regular basis according to agency healthcare setting practice and policy, Level III

Discussion: Patients usually respond to therapy very quickly; within the first 3–5 days their wound bed and periwound skin should show noticeable changes. These changes will include reduced size (length, width and depth), diminished periwound oedema, increased granulation tissue, less drainage, less slough or eschar as well as less pain suffered by the patient. Consistent and reliable wound assessment remains

a clinical challenge for wound care clinicians. A wound assessment standard needs to be identified, consistently done and documented in the patient record (9,10,14,15).

If wound closure is the goal and the wound is not reduced by 20–40% after 2–4 weeks of therapy, despite efforts to address the underlying causes and cofactors, therapy should be discontinued and alternate methods sought, Level IV

Discussion: Once the therapy has begun the wound should be assessed at regular intervals following institutional/agency policies and using a standardised method or tool to determine if the therapy is effective in wound closure. If sinus tracts are present these should be measured and documented as well (10,15).

Wound closure is not always the only endpoint with therapy. The clinician has the option to take the wound to full closure and epithelialisation or until the identified goals or endpoints have been met. Topical pressurised oxygen therapy can improve the wound to a point that it can be treated with conventional methods. Topical pressurised oxygen therapy may be used to achieve goals such as:

- Promoting a granulation wound bed.
- Challenging a wound that is not responding to traditional closure methods.

Expected outcomes

Increased wound oxygenation, through the application of topical pressurised oxygen, results in increased collagen deposition and tensile strength, Level IIa

Discussion: Although a level of hypoxia is normal during the inflammatory phase of wound healing, a chronic hypoxic state is not conducive to tissue healing and can lead to tissue necrosis (11). Adequate tissue oxygenation, as provided by topical pressurised oxygen therapy, promotes the formation of VEGF-2 and FEGF which in turn increases the production of collagen (9,11). Collagen synthesis is dependent on the hydroxylation of proline and lysine, and the increase of pO_2 converts proline residues to hydroxyproline. This process allows the procollagen peptide chains to assume the triple helix configuration. Once the procollagen has assumed the triple helix conformation and has been excreted, the individual collagen fibres are arranged into linear fibrils via cross-linking of lysyl hydroxylase and a final cross-linking between large fibrils. These cross-linkages are ultimately responsible for tensile strength in healed wounds (12).

Topically applied pressurised oxygen alters angiogenesis-related growth factor expression in wound fluids from chronic diabetic foot ulcers that may be consistent with revascularisation and renewed healing, Level IIa

Discussion: Topical pressurised oxygen therapy has shown a consistent and persistent elevation in the expression of biomarkers VEGF and fibroblast growth factor (FGF)-2 throughout the therapy. Both VEGF and FGF-2 promote epithelialisation and capillary neoangiogenesis. These biomarkers, as measured by Scott, quantify therapeutic angiogenesis, indicating evidence of renewed activation of dormant cells in chronic wounds and therefore promote healing (11,12).

A low recurrence rate may be expected in venous leg ulcers and diabetic foot ulcers following topical pressurised oxygen therapy, Level III

Discussion: Topical pressurised oxygen therapy promotes epithelialisation and capillary neoangiogenesis, leading to the formation of higher collagen tensile strength during wound healing. This in turn has shown to reduce scarring and risk of ulcer recurrence. Blackman *et al*. showed no recurrence after 24 months in either the control group or the group that received topical pressurised oxygen therapy. Tawfick *et al*. showed after 36 months, that 8 of the 13 healed ulcers in the control group recurred compared to none of the 37 healed ulcers in the group that received topical pressurised oxygen therapy (7,15).

Topical pressurised oxygen therapy may reduce wound-related pain in venous leg ulcers, Level III

Discussion: The oscillating cyclical nature of the therapy is thought to assist in removing the interstitial oedema in the tissue, relieving the pain associated with venous stasis and the extreme tensions placed on the tissues. Patients

indicated their pain levels fell from eight to three on the pain scale upon commencement of the therapy (15).

Resources

Education needs to be provided to patients, caregivers and healthcare providers regarding the purpose and process of using topical pressurised oxygen therapy, Level IV

Discussion: Once an order is obtained for the therapy and its duration, healthcare professionals, personal support workers (PSWs) as well as patients and their family members can be instructed on how to set-up and apply topical pressurised oxygen therapy. Selection for who may administer the therapy must be based on the healthcare policy and procedures. The identified individual(s) must receive training on the equipment and its use from a designated distributor employee or designate. Healthcare professionals need to be skilled in providing accurate follow-up for wound assessment and documentation as well as post-treatment dressing application and care (10).

In order to support patient and caregiver understanding and adherence to treatment regimens, several strategies can be used in combination:

1. Emphasise the value of the patient's regimen and the positive effects of adherence.
2. Create a patient regimen that is simple – with simple, clear instructions.
3. Listen to the patient and customise the regimen to his/her lifestyle.
4. Enlist support from the patient's family, friends and community services when needed.

Preliminary studies have shown that topical pressurised oxygen therapy has the potential for cost savings, Level IV

Discussion: Tawfick and Sultan showed at 12 weeks that 80% of the venous leg ulcers were closed in the topical pressurised oxygen therapy group compared with 35% closure in control group. The median time to full closure for all ulcers was 45 days for topical pressurised oxygen therapy group versus 182 days in control group. Fourteen of 17 (82·4%) ulcers in the topical pressurised oxygen therapy group closed, with a median average of 56 days. 5 of 11 (45·45%) of the ulcers closed in the control group, with median average of 93 days (7,15).

In contrasting the topical pressurised oxygen therapy group with the control group, cost savings are evident in the areas of physician visits, debridement, dressing, antibiotics and hospitalisations – and also in possible amputations.

According to Blackman *et al*. 'The significant differences in treatment outcomes confirm the potential in the benefits of topical pressurised oxygen therapy in the management of difficult to heal diabetic foot ulcers (DFUs). Clinical efficacy and cost-effectiveness studies are warranted.'

Safety and precautions

Protocols for oxygen safety must be followed when topical pressurised oxygen therapy is in use

Discussion: Because oxygen is a non flammable and non explosive gas it does not burn; however, it does support combustion. Any material that will burn in air will ignite more readily in an oxygen-enriched environment. According to the Ontario Ministry of Health and Long-Term Care, oxygen users must take precautions when using oxygen. Keep oxygen systems away from sources of heat or open flame. Patients, caregivers, family or visitors should not smoke or let anyone else smoke in the area where oxygen is in use. Patients need to be reminded that smoking is not only a health risk but it eliminates the benefits of oxygen therapy. A warning sign must be posted wherever oxygen is in use; as well the local fire department should be notified there is oxygen in the home. Oxygen needs to be stored in a well-ventilated non confined area. Frost injuries to the skin can occur if filling is not done correctly, so the manufacturers' recommendations must be followed to ensure the safe and effective use of this therapy. Vaseline or other petroleum products containing grease or oils, petroleum jelly, alcohol or flammable liquids that can cause oxygen to be flammable should not be on or near an oxygen system (17).

FURTHER STUDIES

Further studies are required to determine if topical pressurised oxygen therapy is indicated for the treatment of acute post-surgical wounds, skin grafts and flaps, and burns. Identified endpoints such as reduced peripheral

Key Points

- further studies are required to determine if topical pressurised oxygen therapy is indicated for the treatment of acute postsurgical wounds, skin grafts and flaps, and burns
- identified endpoints such as reduced peripheral oedema and decreased bacterial burden also require further research
- in addition, randomised controlled trials (RCTs) would be beneficial to increase the evidence around the use and effectiveness of topical pressurised oxygen therapy and to establish optimal parameters for use
- endpoints identifying the patient's perspective are needed to show improved quality of life
- there have been no specific cost effectiveness studies completed

Key Points

- the review undertaken in this initiative used a recognised methodology for systematically exploring the evidence around topical pressurised oxygen therapy to identify statements that are not only evidence-based but also agreed upon by experts in the field
- the result is this document, which provides a standard by which clinicians and decision/policy makers can make an informed decision on the use of topical pressurised oxygen therapy regarding the appropriateness of implementation into practice
- it also identifies where further research is required to provide a more complete picture regarding the effective use of topical pressurised oxygen therapy

oedema and decreased bacterial burden also require further research.

In addition, randomised controlled trials (RCTs) would be beneficial to increase the evidence around the use and effectiveness of topical pressurised oxygen therapy and to establish optimal parameters for use. Current studies show the efficacy of the therapy in DFUs and venous leg ulcers (VLUs). However, there is variation in protocols and dosing methods, and therefore RCTs are warranted to improve understanding of the parameters for use.

There have been no studies found that show improved quality of life for patients receiving topical pressurised oxygen therapy. It has been implied that if topical pressurised oxygen therapy can close wounds more quickly and efficiently, the patients can retain their limbs and remain ambulatory and can be a part of the contributing work force. However, endpoints identifying the patient's perspective are needed to show improved quality of life.

Although studies have suggested that topical pressurised oxygen therapy is cost effective there have been no specific cost effectiveness studies completed.

CONCLUSION

Clinicians addressing wound care concerns are often bombarded by therapies claiming to heal wounds. The review undertaken in this initiative used a recognised methodology for systematically exploring the evidence around topical pressurised oxygen therapy to identify statements that are not only evidence-based but also agreed upon by experts in the field. The result is this document, which provides a standard by which clinicians and decision/policy makers can make an informed decision on the use of topical pressurised oxygen therapy regarding the appropriateness of implementation into practice. It also identifies where further research is required to provide a more complete picture regarding the effective use of topical pressurised oxygen therapy.

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