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

MORE LIKELY TO HEAL A DFU IN 12 WEEKS

LOWER RECURRENCE AT 12 MONTHS

STUDY DESCRIPTION

A state-of-the-art, level 1A evidence, Randomized Controlled Trial (RCT) demonstrating the efficacy of multi-modality cyclical pressure Topical Wound Oxygen (TWO2) therapy in healing and reducing recurrence of Diabetic Foot Ulcers (DFU).

A Group Sequential Design was utilized for the study with three predetermined analyses and hard stopping rules at 73, 146 and 220 on completing a 12-week treatment phase (p < 0.022 at each analysis point). All data analysis utilized an Intention-to-treat (ITT) approach.

Patients meeting eligibility criteria were enrolled into a 2-week run-in with defined optimal standard-of-care (SOC) Only hard-to-heal ulcers were included in the study, 25% of run-in patients were excluded prior to randomization due to achieving 30% wound area reduction on SOC alone.

Kaplan-Meier Wound Closure Estimates

Logrank test (Chi², 1dif)=6.75, p=0.009

Proportion Closed (%) vs. Time (Days)
RESULTS
TWO2 was shown to be 6 times more likely to heal a DFU at 12 weeks compared to optimal SOC after adjusting for ulcer severity.

TWO2 demonstrated more durable healing with a 6 times lower recurrence rate compared to optimal SOC with only 6.7% of Active TWO2 vs 40% of Sham TWO2 ulcers recurring at 12 months.

The wound care–focused QOL index improved for patients whose ulcers healed with Active TWO2 in all functional domains, with the greatest improvement seen in the Well-Being component that improved 90-fold.

Primary Endpoint of Ulcers 100% Healed at 12 Weeks:
Active TWO2 = 41.7% vs Sham TWO2 = 13.5%
(Pearson Chi^2 = 7.27, P = 0.007)

Likely Healing Outcome at 12 Weeks:
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
Odds ratio (OR) increased to 6.00 (97.8% CI 1.44, 24.93), P=0.004

For the patients with larger open ulcers at the end of the 12-week active phase, the mean reduction in ulcer area from baseline was 4.12 cm for Active TWO2 compared with a 1.34 cm increase for the Sham TWO2

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

TWO2 therapy was shown to be safe, without complications, and provided more durable healing for those who had wound closure compared to optimal Standard care alone.

Additionally, TWO2 can be easily administered by the patient at home without the expense and difficulties of daily travel to a specialized center and can also be combined with other advanced wound care modalities.
A Multinational, Multicenter, Randomized, Double-Blinded, Placebo-Controlled Trial to Evaluate the Efficacy of Cyclical Topical Wound Oxygen Therapy (TWO2) in the Treatment of Chronic Diabetic Foot Ulcers: The TWO2 Study

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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.
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, H2O2 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 PO2 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 biologic 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 boards 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, in a randomized study of topical oxygen, demonstrated better healing in DFU patients after 90 days (90% vs. 40%) compared with the control group (27). 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 analyses 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 analyses 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 $\geq 1$ cm$^2$ and $< 20$ cm$^2$ post-debridement. All ulcers included were to be between 4 weeks and 1 year in duration and 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 TcPO2 > 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 randomly assigned double-blind into either the active (SC+TWO2) or sham (SC+Sham) study arms.

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 solely 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>
<thead>
<tr>
<th>Table 1—Inclusion/exclusion criteria</th>
<th>Inclusion criteria</th>
<th>Exclusion criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males and females aged between 18 and 89 years</td>
<td>Evidence of gangrene on any part of affected limb</td>
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<tr>
<td>Documented diagnosis of type 1 or 2 diabetes</td>
<td>Documented evidence of osteomyelitis on any part of affected limb</td>
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<tr>
<td>Foot ulcer at or below ankle with duration &gt; 4 weeks to &lt; 1 year</td>
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<tr>
<td>• If the index ulcer is postamputation, date of surgery must be &gt; 30 days</td>
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<tr>
<td>• If &gt; 1 ulcer is present, largest is considered as the study index ulcer</td>
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<tr>
<td>• Index ulcer must be ≥ 1 cm from any other ulcers present on the foot</td>
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<tr>
<td>Ulcer size ≥ 1 and ≤ 20 cm² after debridement at start of run-in period</td>
<td>Known immune insufficiency</td>
<td></td>
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<tr>
<td>Ulcer of UTC grade 1A, 1B, 1C, 1D, 2A, 2B, 2C, or 2D</td>
<td>Active treatment for malignancy (not specific to study limb)</td>
<td></td>
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<tr>
<td>ABI ≥ 0.7 with a TcPO2 &gt; 30 mmHg, skin perfusion &gt; 30 mmHg, toe pressure &gt; 30 mmHg, or Duplex ultrasound with biphasic waveforms below the knee</td>
<td>Chronic steroid use or immunosuppressive agents within the last 3 months or anticipated to require them during the duration of the study</td>
<td></td>
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<tr>
<td>No planned revascularization procedure or vascular surgery within the last or next 30 days</td>
<td>Subject participated in another investigational device, drug, or biological trial within last 30 days</td>
<td></td>
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<tr>
<td>Subject and caregiver willing and able to comply with all specified care and visit requirements</td>
<td>Index ulcer exhibits signs of severe clinical infection that requires hospitalization or immediate surgical intervention</td>
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<tr>
<td>Subject has a reasonable expectation of completing the study</td>
<td>Subject is pregnant at the time of screening</td>
<td></td>
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<tr>
<td>Subject completed 2-week run-in period with &lt; 30% wound size reduction</td>
<td>Subject has had a deep vein thrombosis within the last 30 days</td>
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<tr>
<td>Subject has received growth factor therapy, autologous platelet-rich plasma gel, bilayered cell therapy, dermal substitute, extracellular matrix, etc., within the screening period</td>
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</tbody>
</table>
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 (Stata Corp, 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 HbA1c (%). 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 [CONSORT diagram]).

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 [baseline characteristics]).

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

### Table 2—Baseline characteristics

<table>
<thead>
<tr>
<th></th>
<th>Sham TWO2 (n = 37)</th>
<th>Active TWO2 (n = 36)</th>
<th>Total (n = 73)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years, mean (SD)</td>
<td>61.9 (9.5)</td>
<td>64.6 (10.3)</td>
<td>63.3 (9.9)</td>
<td>0.21</td>
</tr>
<tr>
<td>Sex, male (%)</td>
<td>31 (84)</td>
<td>32 (89)</td>
<td>63 (86)</td>
<td>0.53</td>
</tr>
<tr>
<td>Race, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White/Hispanic</td>
<td>24 (65)</td>
<td>26 (72)</td>
<td>50 (68.5)</td>
<td></td>
</tr>
<tr>
<td>Black</td>
<td>5 (14)</td>
<td>5 (14)</td>
<td>10 (14)</td>
<td>0.90*</td>
</tr>
<tr>
<td>Asian</td>
<td>1 (2.7)</td>
<td>2 (5.6)</td>
<td>3 (4.1)</td>
<td></td>
</tr>
<tr>
<td>American Indian</td>
<td>1 (2.7)</td>
<td>0 (0)</td>
<td>1 (1.4)</td>
<td></td>
</tr>
<tr>
<td>Not reported</td>
<td>6 (16.2)</td>
<td>3 (8.3)</td>
<td>9 (12.3)</td>
<td></td>
</tr>
<tr>
<td>Type 2 diabetes, n (%)</td>
<td>33 (89)</td>
<td>32 (89)</td>
<td>65 (89)</td>
<td>0.97</td>
</tr>
<tr>
<td>BMI (kg/m²), mean (SD)</td>
<td>31.2 (7.6)</td>
<td>30.8 (5.9)</td>
<td>31 (6.8)</td>
<td>0.85</td>
</tr>
<tr>
<td>Wound area (cm²), mean (SD)</td>
<td>3.22 (2.54)</td>
<td>3.02 (2.66)</td>
<td>3.13 (2.57)</td>
<td>0.74</td>
</tr>
<tr>
<td>Ulcer duration (days), mean (SD)</td>
<td>6.85 (4.18)</td>
<td>6.22 (2.85)</td>
<td>6.54 (3.55)</td>
<td>0.45</td>
</tr>
<tr>
<td>Wound classification, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UTC grade IA</td>
<td>27 (73)</td>
<td>20 (56)</td>
<td>47 (64)</td>
<td></td>
</tr>
<tr>
<td>UTC grade IB</td>
<td>2 (5.4)</td>
<td>1 (2.8)</td>
<td>3 (4.1)</td>
<td></td>
</tr>
<tr>
<td>UTC grade IC</td>
<td>2 (5.4)</td>
<td>1 (2.8)</td>
<td>3 (4.1)</td>
<td></td>
</tr>
<tr>
<td>UTC grade IIA</td>
<td>4 (10.8)</td>
<td>9 (25)</td>
<td>13 (17.8)</td>
<td>0.03**</td>
</tr>
<tr>
<td>UTC grade IIB</td>
<td>0 (0)</td>
<td>1 (2.8)</td>
<td>1 (1.4)</td>
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</tr>
<tr>
<td>UTC grade IIC</td>
<td>2 (5.4)</td>
<td>4 (11.1)</td>
<td>6 (8.2)</td>
<td></td>
</tr>
<tr>
<td>Neuropathic foot, n (%)</td>
<td>29 (78)</td>
<td>28 (78)</td>
<td>57 (78)</td>
<td>0.95</td>
</tr>
<tr>
<td>Charcot deformity, n (%)</td>
<td>3 (8.1)</td>
<td>1 (2.8)</td>
<td>4 (5.4)</td>
<td>0.32</td>
</tr>
<tr>
<td>Ulcer location, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dorsal foot</td>
<td>5 (13.5)</td>
<td>8 (22.2)</td>
<td>13 (17.8)</td>
<td></td>
</tr>
<tr>
<td>Leg below malleoli</td>
<td>4 (10.8)</td>
<td>1 (2.8)</td>
<td>5 (6.8)</td>
<td>0.32</td>
</tr>
<tr>
<td>Pedal foot</td>
<td>22 (59.5)</td>
<td>18 (50)</td>
<td>40 (54.8)</td>
<td></td>
</tr>
<tr>
<td>Toe</td>
<td>6 (16.2)</td>
<td>9 (25)</td>
<td>15 (20.5)</td>
<td></td>
</tr>
<tr>
<td>Previous history of lower-extremity amputation, n (%)</td>
<td>8 (21.6)</td>
<td>17 (47.2)</td>
<td>25 (34.3)</td>
<td>0.02</td>
</tr>
<tr>
<td>Comorbidities, n (%)</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Hypertension</td>
<td>30 (81)</td>
<td>28 (78)</td>
<td>58 (79)</td>
<td>0.73</td>
</tr>
<tr>
<td>Cardiovascular disease</td>
<td>9 (24.3)</td>
<td>13 (36.1)</td>
<td>22 (30.1)</td>
<td>0.27</td>
</tr>
<tr>
<td>PAD</td>
<td>4 (10.8)</td>
<td>10 (27.8)</td>
<td>14 (19.2)</td>
<td>0.07</td>
</tr>
<tr>
<td>Venous disease</td>
<td>1 (2.7)</td>
<td>2 (5.6)</td>
<td>3 (4.1)</td>
<td>0.54</td>
</tr>
<tr>
<td>Renal disease</td>
<td>6 (16.2)</td>
<td>10 (27.8)</td>
<td>16 (21.9)</td>
<td>0.23</td>
</tr>
<tr>
<td>Neurologic disease</td>
<td>31 (83.8)</td>
<td>28 (77.8)</td>
<td>59 (80.8)</td>
<td>0.52</td>
</tr>
<tr>
<td>Peripheral edema</td>
<td>1 (2.7)</td>
<td>3 (8.3)</td>
<td>4 (5.4)</td>
<td>0.29</td>
</tr>
<tr>
<td>Hyperlipidemia</td>
<td>25 (67.6)</td>
<td>23 (63.9)</td>
<td>48 (65.8)</td>
<td>0.74</td>
</tr>
<tr>
<td>Smoker, n (%)</td>
<td>10 (27)</td>
<td>13 (36)</td>
<td>23 (31.5)</td>
<td>0.41</td>
</tr>
<tr>
<td>Peripheral arterial circulation parameters</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Mean ABI (SD)</td>
<td>1.00 (0.23)</td>
<td>1.07 (0.23)</td>
<td>1.03 (0.23)</td>
<td>0.20</td>
</tr>
<tr>
<td>Mean systolic blood pressure (mmHg, mean (SD)</td>
<td>83.00 (32.75)</td>
<td>84.50 (30.55)</td>
<td>83.77 (30.63)</td>
<td>0.84</td>
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<tr>
<td>Blood work values, mean (SD)</td>
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<tr>
<td>Prealbumin, μmol/L</td>
<td>4.29 (1.45)</td>
<td>4.44 (0.93)</td>
<td>4.36 (1.18)</td>
<td>0.61</td>
</tr>
<tr>
<td>CRP, nmol/L</td>
<td>140 (173)</td>
<td>65.7 (96.2)</td>
<td>99.6 (139)</td>
<td>0.05</td>
</tr>
<tr>
<td>Creatinine, μmol/L</td>
<td>105.2 (30.1)</td>
<td>113.2 (81.3)</td>
<td>108.7 (61)</td>
<td>0.57</td>
</tr>
<tr>
<td>HbAlc, %</td>
<td>8.14 (1.49)</td>
<td>8.43 (1.75)</td>
<td>8.25 (1.64)</td>
<td>0.46</td>
</tr>
<tr>
<td>HbAlc, mmol/mol</td>
<td>65 (16.3)</td>
<td>69 (19.1)</td>
<td>67 (17.9)</td>
<td>0.46</td>
</tr>
</tbody>
</table>

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.

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

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 [χ² (1df) = 6.13, P = 0.013].

### Wound Area Reduction

For 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) cm² for the active arm compared with 0.40 (1.75) cm² for the sham arm [t (df) = 2.12 (35), P = 0.041].

For the patients with larger open ulcers >4 cm² at the end of the active phase, the mean (SD) absolute reduction in ulcer area from baseline was 4.12 (1.51) cm² for the active arm compared with a 1.34 (1.18) cm² 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
diabetes care

arm.

index limb amputations (8%) in the sham in the active arm compared with three index limb amputations (5%) occurred related adverse events reported. Two of the time.

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 diabetic foot ulcers 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 4.5-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.

![Figure 2](image-url) — Kaplan-Meier curve showing the separation between study groups throughout the 12-week trial.

Figure 2

Partial responders. The greatest improvement was seen for the well-being 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 the sham arm —0.1 (16.9) [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...
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 diabetic foot ulcer 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.

Table 3—Summary of the results: ITT analysis

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Sham TWO2 (n = 37)</th>
<th>Active TWO2 (n = 36)</th>
<th>Pearson ( \chi^2 ) or OR or HR (97.8% CI), ( P ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Primary outcome</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ulcers completely healed at 12 weeks, n (%)</td>
<td>5 (13.5)</td>
<td>15 (41.7)</td>
<td>( \chi^2 7.27 ) (1df), ( P = 0.007 ) OR 4.57 (1.19, 17.57), ( P = 0.010 ) HR 3.64 (1.11, 11.94), ( P = 0.013 )</td>
</tr>
<tr>
<td>By randomized treatment group, univariate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>After adjustment for UT grade</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Margin of effect/relative performance %</td>
<td>68/309</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Secondary outcomes</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Healing durability</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ulcer recurrence at 12 months, n (%)</td>
<td>2 (40.0)</td>
<td>1 (6.7)</td>
<td>( P = 0.070 )</td>
</tr>
<tr>
<td>Ulcers closed at 12 months, n (%)</td>
<td>10 (27)</td>
<td>20 (56)</td>
<td>( P = 0.013 )</td>
</tr>
<tr>
<td>Margin of effect/relative performance %</td>
<td>52/207</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Healing trajectories</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absolute change in ulcer area over 12 weeks, cm(^2)</td>
<td>0.40 (1.75)</td>
<td>1.97 (2.75)</td>
<td>( P = 0.041 )</td>
</tr>
<tr>
<td>Absolute change in ulcer area in ulcers (&gt;4) cm(^2) over 12 weeks, cm(^2)</td>
<td>-1.34 (1.18)</td>
<td>4.12 (1.51)</td>
<td>( P = 0.021 )</td>
</tr>
<tr>
<td>Time to complete wound closure, weeks</td>
<td>6.3 (1.9)</td>
<td>8.2 (4.2)</td>
<td>( P = 0.350 )</td>
</tr>
<tr>
<td>QOL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CWIS well-being improvement between baseline and week 12</td>
<td>-0.1 (16.9)</td>
<td>9.1 (13.9)</td>
<td>( P = 0.033 )</td>
</tr>
<tr>
<td>CWIS social life improvement between baseline and week 12</td>
<td>4.1 (12.4)</td>
<td>7.9 (16.9)</td>
<td>( P = 0.340 )</td>
</tr>
<tr>
<td>CWIS physical symptom improvement between baseline and week 12</td>
<td>4.6 (11.8)</td>
<td>12.1 (23.2)</td>
<td>( P = 0.130 )</td>
</tr>
<tr>
<td>Index limb amputations, n (%)</td>
<td>3 (8)</td>
<td>2 (5)</td>
<td>( P = 0.668 )</td>
</tr>
<tr>
<td>T202 therapy and off-loading compliance</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Used T202 therapy device 5 days/week, 90 min/day, n (%)</td>
<td>35 (96)</td>
<td>34 (94)</td>
<td>( P = 0.978 )</td>
</tr>
<tr>
<td>Used off-loading device (&gt;75)%, n (%)</td>
<td>36 (99)</td>
<td>35 (97)</td>
<td>( P = 0.984 )</td>
</tr>
<tr>
<td><strong>Safety analysis</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Incidence of serious adverse events</td>
<td>10</td>
<td>10</td>
<td>( P = 0.943 )</td>
</tr>
<tr>
<td>Wound infection</td>
<td>2</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Osteomyelitis</td>
<td>5</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Hypoglycemic event</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Urinary tract infection</td>
<td>0</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Significant necrotic tissue</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Cardiovascular event</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>UTC grade 2 ulceration</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Severe maceration/dermatitis</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Pneumonia</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Incidence of adverse events</td>
<td>8</td>
<td>8</td>
<td>( P = 0.950 )</td>
</tr>
<tr>
<td>UTC grade 1 ulceration</td>
<td>0</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Ulcer decline</td>
<td>0</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Minor infection</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Minor osteomyelitis</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Minor necrotic tissue</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Cellulitis</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Swelling/edema</td>
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<td>0</td>
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</tr>
<tr>
<td>Maceration</td>
<td>2</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Dermatitis</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Contusion</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Incidence of adverse device events</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

Data are means (SD) unless otherwise indicated. Boldface type indicates significant differences.
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 AOIT, 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 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 received 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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27. Aburto I, Frye C. A randomized controlled trial to evaluate different treatment regimes with topical wound oxygen (TWO2) on chronic wounds. Oral presentation at the 6th International Symposium on Diabetic Foot, 11–14 May 2011, Noordwijkhout, the Netherlands.


Technical and Clinical Outcome of Topical Wound Oxygen in Comparison to Conventional Compression Dressings in the Management of Refractory Nonhealing Venous Ulcers

Wael A. Tawfick, MRCSI¹, and Sherif Sultan, MD, FRCS, EBQS-VASC, FACS¹,²

Abstract
Topical wound oxygen (TWO²) 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 TWO² 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 TWO² and 61% in patients managed with CCD. At 12 weeks, 76% of the TWO²-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 TWO² 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 TWO²-healed ulcers. The TWO² is effective and valuable in managing RVU. The TWO² 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 ulceration¹ 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 (TWO²). 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 factors¹⁸,¹⁹ and promotes leukocyte function with enhanced bactericidal activity.²⁰-²⁵

Aim and Objectives
We aim to assess the technical and clinical outcome of using TWO² and conventional compression dressings (CCDs) in chronic refractory venous ulceration (RVU).

We previously published our experience in the use of TWO² in chronic RVU.²⁶ In this current study, we aimed to examine the mid-term efficacy of TWO² 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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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 C6s 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 HyperBox (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 TWO2. 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

**TWO2 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 day33 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.
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.

Of the 67 ulcers, 24 ulcers were MRSA positive in the TWO2 group, while 19 of 65 were MRSA positive in the CCD group.

Abbreviations: CCD, conventional compression dressings; F, female; M, male; MRSA, methicillin-resistant Staphylococcus aureus; TWO2, topical wound oxygen.

Table 1. Demographics

<table>
<thead>
<tr>
<th>Demographics</th>
<th>TWO2, n</th>
<th>CCD, n</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of ulcers</td>
<td>67</td>
<td>65</td>
<td>.693b</td>
</tr>
<tr>
<td>Age (mean/range)</td>
<td>69.34 years (range = 46-85 years)</td>
<td>67.78 years (range = 44-88 years)</td>
<td>.447c</td>
</tr>
<tr>
<td>Gender, M: F</td>
<td>38: 29</td>
<td>35: 30</td>
<td>.425c</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>n = 21</td>
<td>n = 18</td>
<td>.554c</td>
</tr>
<tr>
<td>Smoking</td>
<td>n = 5</td>
<td>n = 2</td>
<td>.291c</td>
</tr>
<tr>
<td>Hypertension</td>
<td>n = 30</td>
<td>n = 31</td>
<td>.628c</td>
</tr>
<tr>
<td>MRSA positive</td>
<td>n = 24</td>
<td>n = 19</td>
<td>.386c</td>
</tr>
<tr>
<td>Patient referred for primary amputation</td>
<td>n = 3</td>
<td>n = 0</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: CCD, conventional compression dressings; F, female; M, male; MRSA, methicillin-resistant Staphylococcus aureus; TWO2, topical wound oxygen.

Table 2. Characteristics of the Leg Ulcers

<table>
<thead>
<tr>
<th>Anatomical Distribution</th>
<th>TWO2, n</th>
<th>CCD, n</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medial maleolus</td>
<td>32</td>
<td>30</td>
<td>.406b</td>
</tr>
<tr>
<td>Lateral maleolus</td>
<td>16</td>
<td>17</td>
<td>.574b</td>
</tr>
<tr>
<td>Calf</td>
<td>9</td>
<td>9</td>
<td>.840b</td>
</tr>
<tr>
<td>Shin</td>
<td>10</td>
<td>9</td>
<td>.801b</td>
</tr>
<tr>
<td>Ulcer surface area</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤5 cm²</td>
<td>9</td>
<td>8</td>
<td>.459b</td>
</tr>
<tr>
<td>6 to 10 cm²</td>
<td>10</td>
<td>9</td>
<td>.801b</td>
</tr>
<tr>
<td>11 to 20 cm²</td>
<td>25</td>
<td>28</td>
<td>.538b</td>
</tr>
<tr>
<td>21 to 40 cm²</td>
<td>12</td>
<td>11</td>
<td>.794b</td>
</tr>
<tr>
<td>≥41 cm²</td>
<td>11</td>
<td>9</td>
<td>.715b</td>
</tr>
<tr>
<td>Duration of the ulcer</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 to 3 years</td>
<td>12</td>
<td>11</td>
<td>.794b</td>
</tr>
<tr>
<td>4 to 5 years</td>
<td>23</td>
<td>18</td>
<td>.407b</td>
</tr>
<tr>
<td>6 to 10 years</td>
<td>19</td>
<td>22</td>
<td>.446b</td>
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<tr>
<td>11 to 20 years</td>
<td>9</td>
<td>11</td>
<td>.726b</td>
</tr>
<tr>
<td>Over 20 years</td>
<td>4</td>
<td>3</td>
<td>.874b</td>
</tr>
</tbody>
</table>

Abbreviations: CCD, conventional compression dressings; TWO2, topical wound oxygen.

Table 3. The CEAP Classification

<table>
<thead>
<tr>
<th>CEAP Classb</th>
<th>TWO2, n</th>
<th>CCD, n</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>C6,s</td>
<td>67</td>
<td>65</td>
<td>.186c</td>
</tr>
<tr>
<td>Ep</td>
<td>47</td>
<td>51</td>
<td>.589c</td>
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<tr>
<td>Es</td>
<td>20</td>
<td>14</td>
<td>.531c</td>
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<tr>
<td>Ap</td>
<td>15</td>
<td>20</td>
<td>.769c</td>
</tr>
<tr>
<td>As, p</td>
<td>41</td>
<td>38</td>
<td>.259c</td>
</tr>
<tr>
<td>Pr</td>
<td>46</td>
<td>42</td>
<td>.217c</td>
</tr>
<tr>
<td>Po</td>
<td>4</td>
<td>3</td>
<td>.862c</td>
</tr>
<tr>
<td>Pr,o</td>
<td>17</td>
<td>20</td>
<td>.618c</td>
</tr>
</tbody>
</table>

Abbreviations: CCD, conventional compression dressings; CEAP class, Clinical, Etiological, Anatomical, and Pathophysiological classification; TWO2, topical wound oxygen.

a There was no significant difference between both the groups in the demographies or vascular-related risk factors.

b P value is analyzed using t test

c P values are analyzed using chi-squared test.

Table 1. Demographics

<table>
<thead>
<tr>
<th>Demographics</th>
<th>TWO2, n</th>
<th>CCD, n</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of ulcers</td>
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<tr>
<td>Smoking</td>
<td>n = 5</td>
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<tr>
<td>Hypertension</td>
<td>n = 30</td>
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<td>n = 3</td>
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<td></td>
</tr>
</tbody>
</table>

Abbreviations: CCD, conventional compression dressings; F, female; M, male; MRSA, methicillin-resistant Staphylococcus aureus; TWO2, topical wound oxygen.

a There was no significant difference between both the groups in the CEAP classification.

b Basic CEAP Classification.

c P values are analyzed using chi-squared test.
The TWO2-managed ulcers had a substantially shorter healing time, compared to CCD ulcers, 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 TWO2-managed patients underwent primary varicose vein surgery, while 7 patients (2 TWO2 and 5 CCD) underwent redo-varicose vein surgery.

During the follow-up, 3 of the 51 fully healed TWO2-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 TWO2 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.
The TWO2 circumvents the consequence of a total body hyperbaric chamber, with its drawbacks on eyes, lungs, and ears. 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. This led to the concept of hyperbaric oxygen

**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 TWO2-managed ulcers had a significantly shorter median time to full healing (57 days) compared to 107 days in CCD-managed ulcers (P<.0001). TWO2 indicates topical wound oxygen; CCD, conventional compression dressings.
delivery to the site of tissue loss without the side effects of systemic oxygen toxicity.

Conversely, TWO2 is established on the hypothesis that oxygen diffuses through tissue at a depth of 30 to 50 μm. 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.

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.

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

<table>
<thead>
<tr>
<th>Ulcer Surface Area</th>
<th>TWO2 Median Time to Full Healing</th>
<th>CCD Median Time to Full Healing</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤5 cm²</td>
<td>54 days</td>
<td>87 days</td>
<td>&lt;.0001b</td>
</tr>
<tr>
<td>6 to 10 cm²</td>
<td>60 days</td>
<td>118 days</td>
<td>&lt;.0001b</td>
</tr>
<tr>
<td>11 to 20 cm²</td>
<td>53 days</td>
<td>109 days</td>
<td>&lt;.0001b</td>
</tr>
<tr>
<td>21 to 40 cm²</td>
<td>59 days</td>
<td>113 days</td>
<td>&lt;.0001b</td>
</tr>
<tr>
<td>≥41 cm²</td>
<td>61 days</td>
<td>119 days</td>
<td>&lt;.0001b</td>
</tr>
<tr>
<td>Duration of the ulcer</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 to 3 years</td>
<td>58 days</td>
<td>111 days</td>
<td>&lt;.0001b</td>
</tr>
<tr>
<td>4 to 5 years</td>
<td>63 days</td>
<td>99 days</td>
<td>&lt;.0001b</td>
</tr>
<tr>
<td>6 to 10 years</td>
<td>52 days</td>
<td>102 days</td>
<td>&lt;.0001b</td>
</tr>
<tr>
<td>11 to 20 years</td>
<td>57 days</td>
<td>115 days</td>
<td>&lt;.0001b</td>
</tr>
<tr>
<td>Over 20 years</td>
<td>59 days</td>
<td>n = 0</td>
<td>&lt;.0001b</td>
</tr>
</tbody>
</table>

Abbreviations: CCD, conventional compression dressings; TWO2, topical wound oxygen.

* 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 (98cm² surface area) with thick eschar on medial aspect of the leg.

**Figure 5.** Case 1 after 8 weeks of TWO2 therapy. Ulcer less than 3cm² in the surface area.

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

The TWO2 promotes capillary neoangiogenesis 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.
Diffused oxygen raises the capillary Po2 levels at the wound site, stimulates epithelization, and granulation of new healthy tissue.\textsuperscript{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).\textsuperscript{37,38}

Of the 24 MRSA-positive ulcers in the TWO\textsubscript{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\textsubscript{2} is lethal to anaerobic bacteria and enhances polymorph nuclear function and bacterial clearance.\textsuperscript{20-22} It diminishes neutrophil adherence based on inhibition of β-2 integrin function.\textsuperscript{23} This enlightens us of its potency against MRSA infection. The TWO\textsubscript{2} assists antibiotic dispersion for aminoglycosides, cephalosporins, quinolones, and amphotericin.\textsuperscript{24,25}

Although TWO\textsubscript{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\textsubscript{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\textsubscript{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.\textsuperscript{14-17}

Notwithstanding that the mean Venous Clinical Severity Score\textsuperscript{31,32} was elevated in patients managed with TWO\textsubscript{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\textsubscript{2} therapy.

**Conclusion**

The TWO\textsubscript{2} is prudent, effective, and valuable in managing RVUs without the risks of full body hyperbaric chambers. The TWO\textsubscript{2} slashes the time needed for RVU healing and is successful in pain alleviation, MRSA elimination, and management.

The TWO\textsubscript{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.

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**References**


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...
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 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.

In an effort to address some of these drawbacks, the principle of topical pressurized oxygen administration or topical wound oxygen therapy (TWO2) was introduced in the late 1960s. The approach of topically oxygenating the wound is quite different from HBO. TWO2 does not involve pressures as high as in HBO. Additionally, TWO2 is portable and can be administered in varied care sites, including in the patient’s home. A number of published studies, 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 TWO2, 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 TWO2 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. 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 TWO2 device was available after the initial assessment (there were a total of four devices), the patient was asked to be in the TWO2 arm. If all TWO2 devices were occupied at the first visit of the study participant, or the patient refused daily TWO2 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 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 O2 into an extremity chamber in a cyclical manner. This cycle consists of pressurizing the chamber to 50 mb and then venting the O2 out of the chamber, allowing pressure to reduce toward ambient pressure (5 mb) before re-pressurizing. Treatment consisted of daily 60-minute TWO2 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.
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 TWO2 group withdrew from the study after 81 days and missing >50% of treatments (see Figure 1).

The TWO2 and AMWT groups were similar with respect to age, gender distribution, HbA1c, and ABI. Baseline wound area was significantly larger in the TWO2 than in the control group (mean 4.1 cm² [SD 4.3] versus 1.4 cm² [SD 0.6]; P = 0.02). Wound duration was longer in the TWO2 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 TWO2 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 TWO2 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 TWO2 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 TWO2 therapy or control group.

No treatment-related adverse events were documented in either group.
benefits of TWO2 compared to AMWT.

In this respect, the results of this trial may underestimate the potential for wounds in patients treated with TWO2 in this study were significantly more likely to heal and 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 studies23-27 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 study27 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 TWO2 remain, evidence suggests that TWO2 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 (pO2) in the wound is lower than in healthy tissue; in dermal wounds, pO2 ranges from 0 to 10 mm Hg in the center of the wound to 60 mm Hg at the periphery.6 In contrast, the pO2 in arterial blood is approximately 100 mm Hg.

Oxygen needed for collagen synthesis proceeds in direct proportion to pO2 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 O2 in collagen synthesis has been determined to occur at a pO2 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 pO2 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

Discussion

Overall study results. Wounds in patients treated with TWO2 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 TWO2 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 TWO2 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 TWO2 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
by activation of the plasma membrane-bound NADPH oxidase. Research presented by Hunt\(^1\) 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 ($\text{O}_2^-$) as well as hydrogen peroxide ($\text{H}_2\text{O}_2$). 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.\(^3\)

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 $\text{pO}_2$ in the wound tissue. Fries et al\(^18\) studied the efficacy of topical oxygen in an experimental model involving excisional dermal wounds in pigs. Exposing open dermal wounds to topical oxygen treatment increased superficial wound tissue $\text{pO}_2$. Fries et al used a probe designed to measure superficial $\text{pO}_2$ at 2 mm depth at the center of the wound bed and saw an increase of $\text{pO}_2$ 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\(^35\) 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. $\text{TWO}_2$ 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 $\text{TWO}_2$, the most crucial angiogenesis-related growth factor, VEG-F, increased as much as 20-fold.\(^3\)

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 $\text{TWO}_2$ ($n = 25$; 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.\(^16,21,28-36\) The results obtained in this trial confirm previously published results of using $\text{TWO}_2$ 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, known 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

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**Table 1. Baseline patient and wound characteristics**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Control group</th>
<th>TWO$_2$ group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$N = 11$</td>
<td>$N = 17$</td>
</tr>
<tr>
<td>Age (years)</td>
<td>63.4 (9.6)</td>
<td>62.4 (8.7)</td>
</tr>
<tr>
<td>Gender (male)</td>
<td>8 (72.7%)</td>
<td>12 (70.6%)</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>7.4% (1.2%)</td>
<td>7.3 (1.2)</td>
</tr>
<tr>
<td>Current smoker</td>
<td>0 (0%)</td>
<td>2 (11.8%)</td>
</tr>
<tr>
<td>Ankle-brachial systolic pressure</td>
<td></td>
<td></td>
</tr>
<tr>
<td>index (mm Hg)</td>
<td>1 (0.18)</td>
<td>0.9 (0.21)</td>
</tr>
<tr>
<td>Wound duration before therapy</td>
<td>3.2 (0.4)</td>
<td>6.1 (5.8)</td>
</tr>
<tr>
<td>(months)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wound area (cm$^2$)</td>
<td>1.4 (0.6)$^a$</td>
<td>4.1 (4.3)$^a$</td>
</tr>
<tr>
<td>Wound stage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C II</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>C III</td>
<td>0 (0%)</td>
<td>1 (5.9%)</td>
</tr>
<tr>
<td>D II</td>
<td>7 (63.6%)</td>
<td>5 (29.4%)</td>
</tr>
<tr>
<td>D III</td>
<td>4 (36.4%)</td>
<td>11 (64.7%)</td>
</tr>
<tr>
<td>Received offloading therapy</td>
<td>11 (100%)</td>
<td>17 (100%)</td>
</tr>
<tr>
<td>Planter location of wound</td>
<td>11 (100%)</td>
<td>17 (100%)</td>
</tr>
<tr>
<td>1st metatarsal</td>
<td>10 (91%)</td>
<td>4 (22%)</td>
</tr>
<tr>
<td>3rd metatarsal</td>
<td>1 (10%)</td>
<td>1 (6%)</td>
</tr>
<tr>
<td>5th metatarsal</td>
<td>-</td>
<td>11 (61%)</td>
</tr>
<tr>
<td>Midfoot</td>
<td>--</td>
<td>1 (5%)</td>
</tr>
<tr>
<td>Loss of protective sensation</td>
<td>11 (100%)</td>
<td>17 (100%)</td>
</tr>
<tr>
<td>History of plantar ulceration</td>
<td>10 (90%)</td>
<td>15 (88%)</td>
</tr>
<tr>
<td>Charcot foot</td>
<td>1 (5.9%)</td>
<td></td>
</tr>
</tbody>
</table>

Data are mean (SD) or number of patients (%)

$^aP = 0.05$
wounds showed mild improvement; all TWO2-treated wounds healed within 7 weeks.

Heng et al20 conducted a prospective randomized controlled study utilizing TWO2. Participants included 40 inpatients with 79 necrotic/gangrenous ulcers assigned to TWO2 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 once to three times daily. TWO2 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 TWO2 group, 90% of ulcers healed compared with 22% in the control group.

Heng et al21 also conducted a 3-month prospective cohort study to assess the healing rate and cost-effectiveness of TWO2 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 amputation with subsequent treatment of the skin defect with TWO2. Fifteen (15) patients had 24 wounds, out of which 22 healed in 24 weeks.

Tawfick et al26 recently published the results of an 83-patient parallel observational study comparing TWO2 and conventional compression therapy used in venous ulcer management. After 12 weeks, 80% of TWO2-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 TWO2-managed patients improved and none of the 19 methicillin-resistant Staphylococcus aureus (MRSA)-positive ulcers in the TWO2 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 study27 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 al28 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 TWO2 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, TWO2 has the potential to provide substantial cost savings.

Conclusion
A significant difference in the proportion of DFUs healed was observed between daily TWO2-treated wounds and those managed with advanced wound dressings. TWO2 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 occurrence of healed ulcers was observed in either treatment group. Well-designed RCTs to confirm the efficacy and evaluate the cost-effectiveness of TWO2 are needed.

References
Does Topical Wound Oxygen (TWO₂) Offer an Improved Outcome Over Conventional Compression Dressings (CCD) in the Management of Refractory Venous Ulcers (RVU)? A Parallel Observational Comparative Study

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Submitted 24 August 2008; accepted 31 March 2009

Abstract  Objectives: Topical wound oxygen (TWO₂) may help wound healing in the management of refractory venous ulcers (RVU). The aim of this study was to measure the effect of TWO₂ on wound healing using the primary end-point of the proportion of ulcers healed at 12 weeks. Secondary end-points were time to full healing, percentage of reduction in ulcer size, pain reduction, recurrence rates and Quality-Adjusted Time Spent Without Symptoms of disease and Toxicity of Treatment (Q-TWiST).

Design: A parallel observational comparative study.

Methods: Patients with CEAP C₆, RVU, assessed by duplex ultrasonography, were managed with either TWO₂ (n = 46) or conventional compression dressings (CCD) (n = 37) for 12 weeks or till full healing. Patients were followed up at 3 monthly intervals.

Results: At 12 weeks, 80% of TWO₂ managed ulcers were completely healed, compared to 35% of CCD ulcers (p < 0.0001). Median time to full healing was 45 days in TWO₂ patients and 182 days in CCD patients (p < 0.0001). The pain score threshold in TWO₂ managed patients improved from 8 to 3 by 13 days. After 12-month follow-up, 5 of the 13 healed CCD ulcers...
showed signs of recurrence compared to none of the 37 TWO2 healed ulcers. TWO2 patients experienced a significantly improved Q-TWiST.

Conclusion: TWO2 reduces recurrence rates, alleviates pain and improves the Q-TWiST. We believe it is a valuable tool in the armamentarium of management of RVU.

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**Introduction**

Refractory venous leg ulceration is a common source of morbidity, and reduced quality of life, especially in the elderly population. The prevalence of venous ulcers has been estimated at 0.3% within the UK population, with comparable rates in other countries. There is a probable underestimation of the true extent due to under-reporting.

Venous ulcers are characterized by a cyclical pattern of healing and recurrence, with recurrence rates up to 70% at one year. Venous ulceration places a huge burden on the healthcare system. The cost of managing venous leg ulcers amasses to £400 million sterling per year in the UK. It causes a considerable amount of morbidity amongst patients, with work incapacity, social exclusion and lack of self esteem.

Conventional compression dressings (CCD) are now widely recognised as the main treatment for venous leg ulcers, with the addition of surgical correction of superficial venous reflux to reduce recurrence rates. However, the socio-economic implications of management of RVU, combined with high recurrence rates have stimulated the development of innovative therapies, as Topical Wound Oxygen (TWO2) therapy.

The application of positive pressure oxygen to manage open wounds has been studied extensively for decades, demonstrating promising clinical results. The traditional limitations of a full body hyperbaric chamber have been overcome by an approach that allows the application of topical wound pure oxygen at an appropriate cycled pressure to only the specific wound site. This maximizes the beneficial wound healing effects and minimizes the negative systemic side effects.

The intermittent cycled pressure, under which the TWO2 is delivered, stimulates circulation, reduces oedema and provides a sealed humidified environment essential for healing. TWO2 promotes epithelialisation and capillary neoangiogenesis. This leads to higher tensile strength collagen being formed during wound healing, which reduces scarring and the risk of recurrence.

**Objectives**

This parallel group observational comparative study was aimed at examining the safety and efficacy of TWO2 in managing refractory venous leg ulcers (RVU). We aim to compare the outcome of using TWO2 to that of CCD in chronic RVU.

**Primary end-points**

The primary end-point study is the proportion of ulcers healed at 12 weeks.

**Secondary end-points**

Secondary end-points are time taken for full healing, percentage of reduction in the ulcer size at 12 weeks, MRSA elimination, pain reduction, recurrence rates and Quality-Adjusted Time Spent Without Symptoms of disease and Toxicity of treatment (Q-TWiST).

**Methods**

Ethical approval was obtained from the local research ethics committee. Patients with chronic refractory non-healing venous ulcers, with an ulcer of more than two years duration, were recruited from the vascular unit in a tertiary referral centre. All patients had to have shown no sign of improvement of the ulcer over the past year, despite adequate compliance with appropriate treatment, provided by community based leg ulcer clinics (Table 1).

All patients were managed on an intention to treat basis. They were given the choice to either be managed using CCD or TWO2. Patients were fully briefed on both therapies and treatment was discussed with their primary care physician and local tissue viability nurse. Allocation to treatment was based on patient’s choice. All patients signed an informed consent prior to commencement of therapy.

**Inclusion criteria:**
- Written informed consent
- ≥18 years of age
- Venous ulcer, with normal ankle–brachial index (ABI) ≥0.9 and digital pressures ≥0.7
- Duration of ulcer of more than two years
- No improvement over the past year

**Exclusion criteria:**
- Bed ridden patients
- Ischaemic ulcers
- Diabetic ulcers
- Osteomyelitis
- Presence of gangrene
- Deep venous thrombosis

Patients underwent a venous duplex scan and a full CEAP assessment (Table 1). ABIs and big toe digital pressures were measured. Punch biopsies were taken from all patients.

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

The leg ulcer was swabbed and a sample taken for culture and sensitivity.
Patients were asked to assess the severity of their pain, on a scale from 1 to 10 using the pain numerical rating scale, prior to therapy and repeated every 3 days.

Ulcers were cleaned, debrided, digitally photographed and measured using a Visitrak system (Smith & Nephew Ltd, Hull, UK), to determine the surface area and maximum length and width of the ulcer.

Patients receiving CCD were managed in an outpatient leg ulcer clinic, using Profore® multilayer compression bandage system with underlying non-adherent Profore® Wound Contact Layer (WCL) dressings (Profore® by Smith & Nephew Ltd, Hull, UK). Dressings were applied by a tissue viability nurse, supervised by the treating physician. Dressings were changed, depending on the amount of exudate, from one to three times per week, after cleaning, debriding and re-measuring the wound.

TWO₂ patients were managed in an inpatient setup, as oxygen was delivered from piped oxygen wall outlets. During treatment sessions, patients were seated, with the affected limb extended and placed in the AOTI Hyper-Box™ (AOTI Ltd, Galway, Ireland) for 180 min twice daily under pressure of 50 mbar (Fig. 1). Oxygen was supplied at 10 l/min with continuous humidification. Between sessions, the limb was left exposed, with no dressings. Patients were allowed to leave the ward or hospital between treatment sessions, if they desired, during which the ulcer was temporarily covered with a non-adherent WCL dressing and gauze bandage, until they returned. No compression was applied. Wounds were cleaned, debrided and re-measured twice per week.42,43

Treatment was continued until full ulcer healing or for 12 weeks, whichever sooner. When full healing was achieved, patients from both treatment arms were commenced on class II elastic stockings. Patients who did not achieve full ulcer healing by 12 weeks, in either treatment arm, were considered failures of treatment. They were managed with CCD and continued to be seen on a weekly basis. Patients were followed up at three monthly intervals following cessation of therapy.

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 Q-TWIST were assessed throughout the treatment and follow-up period.
Survival time was divided into three periods:

- Toxicity (TOX): time spent with toxicity of disease or severe adverse events prior to disease progression.
- TWiST: time spent without symptoms of disease progression or toxicity of treatment.
- Progression (PROG): Time spent with progression of disease. Progression of disease was defined as ulcer recurrence in fully healed ulcers, or increase in ulcer size in ulcers that had not fully healed.

The mean time spent in each of the three periods was determined separately for each treatment group, using the Kaplan–Meier method.

Mean Q-TWiST for each treatment arm was calculated as:

$$ Q\text{-TWiST} = (\mu_{\text{TOX}} \times \text{TOX}) + \text{TWiST} + (\mu_{\text{PROG}} \times \text{PROG}) $$

TOX, TWiST and PROG represented the mean health state duration from Kaplan–Meier analysis; $\mu_{\text{TOX}}$ and $\mu_{\text{PROG}}$ signify the utility coefficients for TOX and PROG, respectively. TWiST was considered to have utility of 1, indicating the best possible quality of life for a patient with RVU.

$\mu_{\text{TOX}}$ and $\mu_{\text{PROG}}$ were weighted using a range of utility scores, to reflect quality of time in each health state, relative to TWiST. Sensitivity analyses were conducted by varying the assigned utilities for TOX and PROG in 0.25 increments across the full range of possible utility weights from 0 (representing poorest health) to 1.

### Statistical analysis

Data were collected and analysed using SPSS 14 software (SPSS Inc, Chicago, Illinois). Continuous variables were compared with the independent sample t test. Categoric proportions were compared using the Chi-Square test. Mann–Whitney U test was performed to compare unpaired, non-parametric data. Time to healing & Q-TWiST were assessed using Kaplan–Meier with Log-rank comparison.

### Results

46 limbs with 46 ulcers were managed using TWO2 therapy. 37 limbs with 37 ulcers were managed using CCD. 63% of the TWO2 patients were men ($n = 29$). 65% of the CCD patients were men ($n = 24$, $p = 0.524$, Table 1).

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

19/46 ulcers were MRSA positive in the TWO2 group, while 17/37 were MRSA positive in the CCD group ($p = 0.251$) (Table 1).

Using the CEAP classification all patients were classified as C6.$^a,^b$

Using the Venous Clinical Severity Score,$^{47–49}$ the mean score in TWO2 patients was 25, and was 23 in CCD patients.

Following commencement of TWO2 therapy, there was an initial latent phase up to five days, where no reduction in surface area was seen. This was followed by a period of rapid improvement, where ulcers reached 70% reduction in surface area. This was followed by a plateau where healing slowed down until either near healing or full healing (Fig. 2).

89% of the TWO2 managed ulcers showed a reduction in surface area by 3 weeks of treatment ($n = 41/46$), compared to 68% of CCD ulcers ($n = 25/37$, $p = 0.016$).

The proportion of ulcers completely healed by 12 weeks was 80% in the TWO2 group ($n = 37/46$) in contrast to 35% of the CCD group ($n = 13/37$, $p < 0.0001$).

The mean reduction in ulcer surface area at 12 weeks was 96% in the TWO2 therapy group, compared to 61% in the CCD group.

| Characteristic of the leg ulcers. 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. |
|-------------------------------------------------|-----------------|-----------------|-----------------|
| **Anatomical distribution** | **TWO2** | **CCD** | **p value** |
| Medial maleolus | $n = 18$ | $n = 14$ | $p = 0.543^a$ |
| Lateral maleolus | $n = 12$ | $n = 11$ | $p = 0.450^a$ |
| Calf | $n = 8$ | $n = 6$ | $p = 0.563^a$ |
| Shin | $n = 8$ | $n = 6$ | $p = 0.563^a$ |

<table>
<thead>
<tr>
<th><strong>Ulcer surface area</strong></th>
<th><strong>TWO2</strong></th>
<th><strong>CCD</strong></th>
<th><strong>p value</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>≤5 cm$^2$</td>
<td>$n = 6$</td>
<td>$n = 6$</td>
<td>$p = 0.459^b$</td>
</tr>
<tr>
<td>6–10 cm$^2$</td>
<td>$n = 7$</td>
<td>$n = 5$</td>
<td>$p = 0.541^b$</td>
</tr>
<tr>
<td>11–20 cm$^2$</td>
<td>$n = 17$</td>
<td>$n = 12$</td>
<td>$p = 0.423^b$</td>
</tr>
<tr>
<td>21–40 cm$^2$</td>
<td>$n = 7$</td>
<td>$n = 7$</td>
<td>$p = 0.437^b$</td>
</tr>
<tr>
<td>≥41 cm$^2$</td>
<td>$n = 9$</td>
<td>$n = 7$</td>
<td>$p = 0.584^b$</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Duration of the ulcer</strong></th>
<th><strong>TWO2</strong></th>
<th><strong>CCD</strong></th>
<th><strong>p value</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>2–3 years</td>
<td>$n = 10$</td>
<td>$n = 9$</td>
<td>$p = 0.492^b$</td>
</tr>
<tr>
<td>4–5 years</td>
<td>$n = 16$</td>
<td>$n = 10$</td>
<td>$p = 0.303^b$</td>
</tr>
<tr>
<td>6–10 years</td>
<td>$n = 12$</td>
<td>$n = 12$</td>
<td>$p = 0.347^b$</td>
</tr>
<tr>
<td>11–20 years</td>
<td>$n = 6$</td>
<td>$n = 5$</td>
<td>$p = 0.600^b$</td>
</tr>
<tr>
<td>Over 20 years</td>
<td>$n = 2$</td>
<td>$n = 1$</td>
<td>$p = 0.582^b$</td>
</tr>
</tbody>
</table>

$^a$ p values are Chi-Square.  
$^b$ p values are Mann–Whitney U.
The median time to full ulcer closure was 45 days in the TWO2 group (95% CI: 39–51), compared to 182 days in the Profore group (95% CI: 162–203, \( p < 0.0001 \)) (Fig. 3).

Within the TWO2 group, the duration the patient had the ulcer and the size of the ulcer, did not affect the healing time. TWO2 managed ulcers had a significantly shorter healing time, compared to CCD ulcers, regardless of the duration of ulcer (\( p < 0.0001 \)) or the size of the ulcer (\( p < 0.0001 \)).

Three of the TWO2 patients were referred to our service for primary amputation following failure of other treatment modalities, including skin grafting. These three ulcers healed completely and none of these patients required amputation.

Three of the TWO2 ulcers showed no signs of healing at 4 weeks. One patient had an ulcer exposing tendons and bone. Histology proved that the other two patients have underlying basal cell carcinoma (\( n = 1 \)) and squamous cell carcinoma (\( n = 1 \)).

32/46 of the TWO2 treated ulcers showed a reverse gradient of healing, where healing commenced from the centre of the ulcer and expanded towards the periphery (Fig. 4). Using the pain numerical rating scale, the pain score threshold in the TWO2 managed patients improved from 8 to 3 by 13 days.

9 of the 19 MRSA positive ulcers in the TWO2 therapy group were MRSA negative after 5 weeks of treatment regardless of closure of the ulcer, compared to none of the 17 MRSA positive ulcers in the CCD group (\( p = 0.007 \)).

No local or systemic complications were encountered in either treatment group.

Patients were followed up for a mean of 12 months. During that period, 2 TWO2 patients underwent varicose vein surgery, while 5 patients (2 TWO2 and 3 CCD) underwent redo-varicose vein surgery.

During follow-up, none of the 37 fully healed TWO2 managed ulcers showed signs of recurrence. In comparison, 5 of the 13 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.

TWO2 patients had a significantly shorter mean TOX (1.5 months), in comparison to CCD patients (6 months, \( p < 0.001 \)). TWO2 patients had a significantly longer mean TWiST (12.5 months), opposed to 4.5 months in CCD patients (\( p < 0.001 \)).

TWO2 patients had no PROG, in contrast to a mean PROG of 3 months for CCD patients (\( p < 0.0001 \)).

TWO2 patients experienced an overall improved Q-TWiST when assigned any utility coefficient, across the full range of possible utility weights. When the utility coefficient assigned was 0.5 the Q-TWiST for TWO2 patients was 13.625 compared to 27 in the CCD group (\( p < 0.0001 \), Table 3).

Discussion

Compression therapy within the setup of a leg ulcer clinic is widely recognised as the main modality for managing venous leg ulcers.\(^{19–22}\) High recurrence rates and the socioeconomic burden of RVU, have motivated the development of alternative therapies as TWO2 therapy.

The first publication on the use of TWO2 was by Fischer in 1969.\(^ {25}\) Fischer noted that lesions became aseptic and enhanced granulation was witnessed two days after TWO2. These findings are similar to our own results. In our study,
Table 3  Quality Time Spent Without Symptoms of Disease and Toxicity of Treatment (Q-TWiST) was significantly improved in TWO2 patients.

<table>
<thead>
<tr>
<th>Time period</th>
<th>TWO2</th>
<th>CCD</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TOX</td>
<td>1.5 months</td>
<td>6 months</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>TWIST</td>
<td>12.5 months</td>
<td>4.5 months</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>PROG</td>
<td>0</td>
<td>3 months</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Q-TWiST</td>
<td>13.625</td>
<td>27</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

however, no improvement was witnessed within the first four to five days of TWO2. This discrepancy in timing of clinical improvement could be attributed to the difference in treatment regimes. While Fischer used a constant pressure of 22 mmHg, the AOTI Hyper-Box\textsuperscript{TM} used in our study cycled the pressure between atmospheric pressure and 50 mbar.

A series of feasibility studies and randomised controlled studies, assessed a mixed aetiology of ulcers and none were dedicated to assess the effect of TWO2 on RVU.\textsuperscript{25–31} We believe our study to be the first study on the use of TWO2 in RVU.\textsuperscript{42,43}

In a prospective randomised study by Heng et al., red granulation tissue was present one week after TWO2.\textsuperscript{32} Heng noted absence of clinical scarring and most ulcers healed within 2–16 weeks. This mimics our findings where healthy granulation tissue was witnessed in the ulcers following four to five days of TWO2.

In both our own study and the Heng study,\textsuperscript{32} positive effects could be found, whereas in a study by Leslie et al. no significant effects could be detected.\textsuperscript{33} The treatment schedule in the Leslie study was short, which could have had an impact on the overall results. Two daily 90-min sessions were applied for 7–14 days, compared to 4-h a day, 4 days a week over 4 weeks in the "positive" Heng study\textsuperscript{32} and 3-h bi-daily, 7 days a week in our study.

In our study, treatment was commenced at 90-min sessions once daily, in the first 5 cases where TWO2 was used. These patients were excluded from this study analysis and are not a subset of the 46 patients managed with TWO2. We noted minimal response within the first 10 days of treatment. Through close monitoring and adjusting our protocol, treatment sessions were increased gradually until reaching 180-min sessions bi-daily, where an adequate response was witnessed and no safety concerns were observed.\textsuperscript{42,43}

During TWO2 therapy sessions, patients endured limb elevation. These patients had their ulcers for a minimum of 2 years(up to 43years), and had already shown no signs of improvement over the past year, despite adequate compliance with treatment. While accepting that this may have assisted in ulcer healing, it would be futile to attribute the improved outcome to limb elevation alone.

In our study, only 35% of ulcers managed with CCD fully healed. Whilst accepting that this is a lower rate than most published studies on this treatment, yet the refractory nature of these ulcers, has to be taken into consideration.

Fischer et al.,\textsuperscript{28} showed reduced rates of infection with TWO2. This depicts our findings, where 9 of the 19 MRSA positive ulcers in the TWO2 group were rendered MRSA negative after 5 weeks of treatment.

Cronje stated that if topical oxygen could increase wound oxygen levels, it would create a reverse gradient, with higher values in the wound than in the periphery.\textsuperscript{50} In our study 69.5% (n = 32/46) of the TWO2 treated ulcers showed reverse gradient of healing. All these ulcers further continued to fully heal with minimal scarring and no recurrence. This could be attributed to topical absorption of oxygen, leading to formation of higher tensile strength collagen.\textsuperscript{36–38}

Despite the fact that the mean Venous Clinical Severity Score\textsuperscript{47–49} was higher in TWO2 patients, yet an improved outcome was witnessed compared to CCD patients.

Ulcers that showed no signs of healing in the TWO2 group, proved to have an underlying cause. One patient had an ulcer exposing tendons and bone. The other two ulcers had underlying malignancy. Since this finding, evidence of mitotic activity was added as an exclusion criterion.

TWO2 patients had a significantly improved Q-TWiST compared to CCD patients, denoting an improved outcome (p < 0.0001). TWO2 patients had a significantly shorter mean period of time with TOX (p < 0.0001). This is attributed to the significantly shorter time to full ulcer closure and higher percentage of ulcers that achieved full healing. TWO2 patients had a significantly longer mean TWIST (p < 0.0001). TWO2 managed patients did not experience any complications from their therapy. There was no recurrence of the ulcers or pain witnessed in the TWO2 patients.

TWO2 patients had no time with PROG, compared to a mean period of 3 months of PROG in CCD patients (p < 0.0001). In the TWO2 group, once healing of the ulcer was achieved, these patients continued to maintain an ulcer free course over a mean period of 12 months of follow-up, with no recurrence of symptoms or progress of disease.

Conclusion

TWO2 is safe and effective in RVU management. It has a superior outcome to CCD, through achieving a shorter healing time, alleviating pain, reducing recurrence rates and improving the Q-TWiST. We believe that TWO2 is a valuable tool in the armamentarium of management of patients with RVU, without the risks of full body hyperbaric chambers.

Following these initial observational findings, a randomised controlled trial is currently underway to further assess the benefits of TWO2 therapy.

Conflict of Interest/Funding

None.

Acknowledgements

The authors would like to acknowledge Sean McGuigan (Medical statistician, Melbourne, Australia, Linde Gas Therapeutics) for his help with the statistical analysis of this study.

The authors would like to thank AOTI Ltd, Galway, Ireland for supplying the Hyper-Box\textsuperscript{TM} and consumables.
References


Nothing to disclose; S. Rowell: Nothing to disclose; R. Urankar: Nothing to disclose.

PS172.

Does Topical Wound Oxygen (TWO2) Offer an Improved Outcome Over Conventional Compression Dressings (CCD) in the Management of Refractory Non-healing Venous Ulcers (RVU)? Three-Year Technical and Clinical Outcome and Midterm Results With Quality-Adjusted Time Spent Without Symptoms of Disease and Toxicity of Treatment (Q-TWiST)

Sherif Sultan, Wael Tawfick. Vascular & Endovascular Surgery, Western Vascular Institute, Galway, Ireland

**Objectives:** TWO2 proposes an option in the management of RVU. Primary endpoint is ulcer healing at 12 weeks and secondary endpoint is Q-TWiST.

**Methods:** 46 ulcers were managed using TWO2 therapy and 37 ulcers with CCD. Demographics and risk factors were similar in both groups. All ulcers were CEAP C6. s.

**Results:** The mean reduction in ulcer surface area at 12 weeks was 96% in the TWO2 therapy group, compared to 61% in the CCD group. At 12 weeks, 80% of TWO2 managed ulcers were completely healed, compared to 35% of CCD ulcers ($p < 0.0001$). Median time to full healing was 45 days in TWO2 patients and 182 days in CCD patients ($p < 0.0001$). 32/46 of TWO2 ulcers showed reverse gradient of healing. 9/19 MRSA positive ulcers managed with TWO2 were rendered MRSA negative after 5 weeks, compared to none of the 17 MRSA positive CCD ulcers. The pain score threshold in TWO2 managed patients improved from 8 to 3 by 13 days. Q-TWiST was significantly longer at 24.25 months for TWO2 and 10.5 months for CCD with $p < 0.0001$. After 36 months follow-up, 8 of the 13 healed CCD ulcers showed recurrence compared to none of the 37 TWO2 healed ulcers. No local or systemic complications were encountered in either treatment group.

**Conclusions:** TWO2 is prudent, effective and valuable in managing RVU up to 36 months and slashes time needed for RVU healing. TWO2 is successful in pain alleviation, MRSA elimination. TWO2 radically degrades recurrence rates and thus enhances the quality of life and has superior Q-TWiST over CCD.

Author Disclosures: S. Sultan: Nothing to disclose; W. Tawfick: Nothing to disclose.

PS174.

A Systematic Review on the Effectiveness of Knee Versus Thigh Length Graduated Compression Stockings in Thromboprophylaxis for Surgical Patients

Mital Y. Desai, Mohammed Shaﬁque Sajid, George Hamilton. Vascular Surgery, Royal Free Hampstead NHS Trust, London, United Kingdom

**Objectives:** To systematically analyze prospective randomized controlled trials on effectiveness of knee (KL) vs thigh length (TL) graduated compression stockings in thromboprophylaxis for surgical patients.

**Methods:** A systematic review of medical literature was undertaken. Prospective randomized controlled trials on postoperative patients of various surgical disciplines were selected according to specific criteria. Data was extracted and analyzed by using statistical package RevMan 5.0. Summated outcome was calculated in form of odds ratio (OR) with 95% confidence interval.

**Results:** Nine trials on 1476 patients were retrieved from electronic databases using standardized medical subject headings. Only three trials on 498 patients qualified for meta-analysis according to inclusion criteria. Both in fixed [OR, 1.55; 95% CI, 0.78 - 3.07; $z = 1.25$; $p = 0.21$] and random [OR, 1.33; 95% CI, 0.44 - 4.06; $z = 0.51$; $p = 0.61$] effects models, KL stockings were as effective as TL stockings for thromboprophylaxis in surgical patients. However, there was significant heterogeneity [Chi2 $= 4.04$, df $= 2$, I2 $= 50\%$] among trials.

**Conclusions:** KL graduated compression stockings may be as effective as TL stockings for the prevention of DVT in surgical patients. For thromboprophylaxis, in surgical patients KL stockings may routinely be used due to parallel efficacy, higher patient compliance and lower cost. However, a major randomized trial is required in order to strengthen the existing evidence.

Author Disclosures: M. Y. Desai: Nothing to disclose; G. Hamilton: Nothing to disclose; M. Sajid: Nothing to disclose.

PS176.

Clinical Outcome Analyses of Radio-Frequency Ablation (RFA) in the Treatment of Incompetent Greater Saphenous Vein (GSV): Differences Between Closure-Plus and ClosureFast Catheters

Natalie Marks, Enrico Ascher, Anil Hingorani, Alexander Shiferon, Kapil Gopal, Daniel Jung, Theresa Jacob. Division of Vascular Services, Maimonides Medical Center, Brooklyn, NY

**Objectives:** The new ClosureFast (CF) catheter has much higher treatment speed as compared to previous ClosurePlus (CP) model. We compared several clinical outcomes after use of both catheters in a large series.

**Methods:** From February 2005 to April 2009 there were 656 consecutive office RFA procedures performed first with CP and later with CF catheters. Postoperative duplex scans (3-7 days) documented technical success (complete obliteration, partial obliteration or full patency
USE OF OXYGEN THERAPIES IN WOUND HEALING

FOCUS ON TOPICAL AND HYPERBARIC OXYGEN TREATMENT
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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
- pO₂: partial pressure of O₂
- 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. 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).

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.

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’.

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>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biofilm</td>
<td>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 defence⁹</td>
</tr>
<tr>
<td>Colonisation</td>
<td>Microbial multiplication in or on the wound without an overt immunological host reaction⁹</td>
</tr>
<tr>
<td>Contamination</td>
<td>Microbial ingress into the wound without growth and division¹⁰</td>
</tr>
<tr>
<td>Endpoint</td>
<td>The occurrence of a disease, symptom, sign, or laboratory abnormality that constitutes the target outcomes of a clinical trial¹¹</td>
</tr>
<tr>
<td>Hyperbaric oxygen therapy (HBOT)</td>
<td>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</td>
</tr>
<tr>
<td>Hypoxia</td>
<td>Inappropriately low availability of molecular oxygen</td>
</tr>
<tr>
<td>Infection</td>
<td>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 symptoms⁹</td>
</tr>
<tr>
<td>Outcome</td>
<td>Documentation of the effectiveness of health-care services and the end results of patient care</td>
</tr>
<tr>
<td>Reactive oxygen species (ROS)</td>
<td>Reactive molecules containing oxygen</td>
</tr>
<tr>
<td>Resource use</td>
<td>The total amount of resources actually consumed, compared against the amount of resources planned for a specific process¹²</td>
</tr>
<tr>
<td>Topical oxygen therapy (TOT)</td>
<td>The administration of oxygen applied topically over injured tissue by either continuous delivery or pressurised systems</td>
</tr>
<tr>
<td>Wound cleansing</td>
<td>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 infection¹⁵</td>
</tr>
</tbody>
</table>
Sufficient availability of molecular oxygen (O₂) 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 O₂ (pO₂) in the wound is below a critical hypoxic threshold level. Hypoxia may result when consumption of O₂ supersedes the delivery of O₂. Poor blood perfusion is traditionally associated with reduced supply of O₂, leading to hypoxia in wounds, which can lead to deficient healing, but the depletion of O₂ resulting from the biological activities within the wound may also contribute significantly to the availability of O₂.¹,¹⁴

**Oxygen supply in wounds**

O₂ delivery in wounds predominately depends on pO₂ 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 O₂. In addition, poor blood circulation may also inhibit the distribution of O₂ in to the wound. Other barriers to appropriate O₂ supply include diffusive constraints due to oedema and O₂ consumption by bacterial biofilm. Also of note, the high metabolic activity present in healing wounds will reduce overall levels of tissue oxygen content.

**3. Role of molecular oxygen in wound healing**

In general, basic need for energy is mainly covered by consumption of O₂ during aerobic respiration. However, a reduction of O₂ 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, O₂ is the most immediate requirement for wound healing in order to reestablish new vessels and connective tissue. O₂ 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 O₂ available for consumption during maturation of collagen fibres and appropriate fibroblast proliferation.

Furthermore, O₂ consumption supports a competent host-response to infection due to the requirement of O₂ for generation of suitable amounts of antimicrobial ROS by phagocytes.¹,¹⁴

**Oxygen consumption during wound healing**

In general, aerobic respiration provides the basic need for energy. However, a reduction of O₂ 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, O₂ is the most immediate requirement for wound healing in order to reestablish new vessels and connective tissue. O₂ 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 O₂ available for consumption during maturation of collagen fibres and appropriate fibroblast proliferation.

**Extra oxygen consumption in wounds with a chronic infection**

Neutrophils are the predominating phagocytes in humans and increased O₂ 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 O₂ consumption is the activation of the phagocytic NADPH-oxidase in order to produce ROS and the ability of NOX-2 to reduce O₂ has been subject to several studies demonstrating the ability to deplete O₂ even when levels are already low.

If the attracted neutrophils manage to successfully
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
Neutrophils surrounding *Pseudomonas aeruginosa* and *Staphylococcus aureus* organised in biofilm may occur. In addition, experimental infection with *Pseudomonas aeruginosa* biofilm has demonstrated increased accumulation of neutrophils in mouse wounds. 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. Such steep oxygen gradients have also been demonstrated in fresh debridement specimens from infected human wounds.

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$_2$. The ability of neutrophils to significantly restrict the availability of O$_2$ is known from other biofilm-associated infections with hypoxia. 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. Likewise, neutrophils are the major consumer of O$_2$ when exposed to *Pseudomonas aeruginosa* biofilm in vitro. 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$_2$ 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, which is typically characterised by intense accumulation of activated neutrophils.

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 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 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). 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 O₂ 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 O₂ therapies, including local O₂ supply or delivery enhancement by haemoglobin, will benefit from the knowledge of the O₂ levels in the proximity of the wound. Measurement of pO₂ 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 O₂ outflow through the skin layers. 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.
Despite almost 50 years of clinical use, the subject of TOT for non-healing wounds remains controversial. 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. This causes both the direct killing of anaerobic bacteria and an enhancement of leukocyte function to address all other pathogens. 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). This results in the prolific structured growth of new blood vessels and the stimulation of collagen synthesis by enhancing fibroblast activity. These factors combined result in better wound bed granulation, strong collagen tissue formation, and wound closure.

**Background**

The first report of TOT was published in 1969 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. 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. In the following years there were inconsistent results in case series and reviews suggesting the putative benefits of administering oxygen topically to chronic wounds.

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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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\textsubscript{2} 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.\textsuperscript{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
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.\textsuperscript{56}

Despite several small case studies indicating beneficial healing for chronic wounds,\textsuperscript{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.\textsuperscript{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).\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.

**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.\textsuperscript{45} The one very poorly conducted RCT that used a similar device has been previously discussed.\textsuperscript{50} Unfortunately, this study is often cited as evidence of the ineffectiveness of TO despite its being underpowered and of too short 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. The cyclical pressure applied with TWO₂ 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. 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 TWO₂ compared with conventional compression dressings (CCD) on wound healing using the primary endpoint of the proportion of ulcers healed at 12 weeks. At 12 weeks, 80% of TWO₂ managed ulcers were completely healed compared with 35% of the CCD-managed ulcers. Median time to full healing was 45 days in the TWO₂ 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 TWO₂ versus CCD in the management of refractory non-healing venous ulcers (RVUs) with a duration of at least two years. 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 TWO₂ and 65 patients (mean age: 68 years) with CCDs for 12 weeks or until full healing. At 12 weeks 76% of the TWO₂ 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 TWO₂ and 0% of patients managed with CCD. Another prospective non-blinded, non-randomised study was conducted to examine the clinical efficacy of TWO₂ therapy in healing patients with severe DFUs referred to a community wound care clinic in Canada. 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 TWO₂ therapy arm and 45.5% in the standard care arm (control) healed completely. Median time to complete healing was of 56 days in the TWO₂ 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 TWO₂ 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.

**Oxygen release through dressings or gels**

Different kinds of products are available, either using the release of pure O₂ embedded in the dressing or releasing O₂ generated by a biochemical reaction in a hydrogel. In the O₂ containing dressings, pure O₂ is embedded, such as in vesicles, and released after the dressing is liquefied by the wound exudate. Continuous O₂ release dressings can be used as secondary dressing and release O₂ 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 O₂ 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 O₂. 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 O₂. The hydrogen peroxide released as a result is thought to diffuse through the dressing and either oxidises iodide ions to free iodine and O₂ or, if it reaches the wound surface, is metabolised to water and O₂. 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 O₂ is believed to create beneficial effects within the wound.³

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 O₂ 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 O₂ 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 O₂ carrier is another approach to topical wound treatment. Haemoglobin augments transport of O₂ by means of facilitated delivery.³⁴ 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 O₂ 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 O₂ 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 \textit{in vivo} in patients with chronic leg ulcers.\textsuperscript{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%.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{79,80}

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 and in England. 

**Application of oxygen species**

Another therapeutic approach using topically applied O₂ 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. 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 O₃, 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 O₂.

These effects are regulated by the basic pH value...

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

<table>
<thead>
<tr>
<th>TOT type</th>
<th>Medical devices</th>
<th>Treatment details</th>
<th>Treatment location</th>
<th>Moist wound environment</th>
<th>GRADE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Higher cyclical pressure oxygen</td>
<td>Aoti Inc., TWO₂</td>
<td>50mbar to 5mbar cycles; Pressure low, &gt;1 bar; Flow rate high; Treatment time: 60–90 minutes; Treatment frequency: 3–7 days</td>
<td>Open wound in chamber or bag</td>
<td>Possible</td>
<td>Grade 18, (RCT, controlled cohort studies, various case series) positive effect shown</td>
</tr>
<tr>
<td>Low constant pressure oxygen in a contained chamber</td>
<td>OxyCare GmbH, O₂TopiCare System</td>
<td>2-5 l/min;&lt;50mbar; Pressure low, &gt;&gt;1 bar; Flow rate: high; Treatment time: 60–90 minutes; Treatment frequency: 3–7 days</td>
<td>Open wound in chamber or bag</td>
<td>Possible</td>
<td>Grade 2C, (1 Interim report on RCT showed no advantage versus sham. Cohort studies, various case series) only weak evidence</td>
</tr>
<tr>
<td>GWR Medical, TO₂</td>
<td></td>
<td>2-5 l/min;&lt;50mbar; Pressure low, &gt;1 bar; Flow rate: high; Treatment time: 60–90 minutes; Treatment frequency: 3–7 days</td>
<td>Open wound in chamber or bag</td>
<td>Possible</td>
<td>Grade 2C, (1 Interim report on RCT showed no advantage versus sham. Cohort studies, various case series) only weak evidence</td>
</tr>
<tr>
<td>Continuous delivery of non-pressurised oxygen (CDO)</td>
<td>Ogenix Inc., EpiFLO</td>
<td>Continuous, slow flow of pure oxygen of 3 ml/hr for 15 days through a cannula to blanket the wound. Pressure low, &lt;1 bar; Flow rate: low; Treatment time: 24 hours; Treatment frequency: 7 days</td>
<td>Occlusive wound dressing</td>
<td>yes</td>
<td>Grade 2C, (1 Interim report on RCT showed no advantage versus sham. Cohort studies, various case series) only weak evidence</td>
</tr>
<tr>
<td>Inotec AMD Ltd., Natrox</td>
<td></td>
<td>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, &lt;1 bar; Flow rate: low; Treatment time: 24 hours; Treatment frequency: 7 days</td>
<td>Occlusive wound dressing</td>
<td>yes</td>
<td>Grade 2C, (1 Interim report on RCT showed no advantage versus sham. Cohort studies, various case series) only weak evidence</td>
</tr>
</tbody>
</table>
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 O₂ solution was 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**      | **Pressure**    | **Flow rate**   | **Treatment time** |
| OxyBand Technologies Inc., OxyBand              | na              | na              | 24 hours        |
| AcryMed/Kimberly Clark, OxygeneSys Continuous   | na              | na              | 24 hours        |
| AcryMed/Kimberly Clark, OxygeneSys On Demand    | na              | na              | 24 hours        |
| Crawford Healthcare Ltd, Oxyzyme                 | na              | na              | 24 hours        |
| SastoMed GmbH, Granulox                          | na              | na              | 24 hours        |
| **Occlusive wound dressing**                    | yes             | yes             |                  |
| **Pressure: na**                                |                 |                 |                  |
| **Flow rate: na**                               |                 |                 |                  |
| **Treatment frequency: 7 days**                 |                 |                 |                  |
| **Use as a foam dressing**                      |                 |                 |                  |
| **Oxygen release for up to 5 days after contact**|                 |                 |                  |
| **with moisture within a simple occlusive wound**|                 |                 |                  |
| **dressing**                                    |                 |                 |                  |
| **Oxygen release for up to 5 days when dressing**|                 |                 |                  |
| **is moistened**                                |                 |                 |                  |
| **Oxysys On Demand**                            |                 |                 |                  |
| **Oxygen release when both layers**              |                 |                 |                  |
| **are attached to each other**                  |                 |                 |                  |
| **Use as a primary dressing**                   |                 |                 |                  |
| **in early stage wound treatment. Oxygen**       |                 |                 |                  |
| **release**                                     |                 |                 |                  |
| **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**                 |                 |                 |                  |
| **SastoMed GmbH, Granulox**                      |                 |                 |                  |
| **Occlusive wound dressing**                    | yes             | yes             |                  |
| **Grade 2B, (1 RCT, cohort studies, various case** |                 |                 |                  |
| **series) only weak recommendation for oxyzyme** |                 |                 |                  |
| **by Nice due to lack of efficacy**              |                 |                 |                  |
| **Grade 1B, (1 RCT, 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**   |                 |                 |                  |
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.54 HOCl has been shown to be effective against a broad range of microorganisms either as stabilised neutral or acidic HOCl-solutions.55 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 5cm² 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.56 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.57 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.
Beyond the most superficial cell layers, there is supposedly no significant topical absorption of O₂. Therefore, for additional O₂ 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 O₂, which is transferred with circulation to all body tissues. If given at sufficiently high pressure, typically 2.2–2.5 ATA, O₂ 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. Generally speaking, there are two types of hyperbaric chambers used worldwide: mono-place, where patients stay alone within small pressurised vessels filled with O₂, 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 pO₂ 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 O₂ 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:

- 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 O₂ radical scavengers, thereby reducing ischemia reperfusion injury.

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

HBOT and bacteria
If pO₂ within the wound exceeds the limits for survival of obligate, facultative anaerobes
and microaerophilic aerobes, the HBOT has a bacteriostatic activity.\textsuperscript{93} During \textit{in vitro} experiments, direct bactericidal effect of high enough $pO_2$ on anaerobic bacteria, i.e. \textit{Clostridium perfringens}, \textit{Bacteroides fragilis}, or \textit{Enterococcus faecalis}, can be observed.\textsuperscript{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.\textsuperscript{95} Moreover, there is a strong synergistic effect of HBOT with at least some antibiotics, including linezolid, vancomycin, teicoplanin, ciprofloxacin and imipenem.\textsuperscript{96–98} We recommend reading the excellent review on HBOT as an anti-infective agent by Cimçıt.\textsuperscript{99}

\section*{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.\textsuperscript{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.

\section*{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.\textsuperscript{102}

\section*{HBOT and genetics}

Interestingly, HBOT modifies gene expressions, this has been noted for genes encoding the IL-8 and ANG expression.\textsuperscript{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.

\section*{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.\textsuperscript{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.

\section*{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 ($p=0.03$).\textsuperscript{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],\textsuperscript{107} VLUs [mean difference 33.00\%, 95\% CI: 18.97–47.03, $p<0.00001$],\textsuperscript{108} mixed arterial and venous wounds [mean difference 61.88\%, 95\%CI: 41.91–81.85, $p<0.00001$]\textsuperscript{108} and recurrent non-healing vasculitic...
wounds not responding to immunosuppressive therapy. 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].

**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. HBOT is generally recognised as a safe procedure and the most often observed side-effects include middle ear barotrauma. 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 has issued specific recommendations ranging from 1A–3C 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 3C)
- 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...
critical ischaemia (see note below), if transcutaneous oxygen pressure readings under hyperbaric conditions (2.5ATA, 100% O₂) 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% O₂). 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 3C)

• 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 3C).
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 Chen116 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 HBOT118–120,121 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 HBOT123–130 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 authors82,131,132 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 patient133 before any collaborative health-care decision being made. Sykes and FitzGerald134 offered the four ‘rights’ of health literacy; right information, right literacy level,
Table 5 Frequently asked questions

<table>
<thead>
<tr>
<th>Pain</th>
<th>HBOT (hyperbaric chamber)</th>
<th>Topical oxygen therapy</th>
<th>Topical oxygen perfusor / chamber</th>
</tr>
</thead>
<tbody>
<tr>
<td>Increase or decrease?</td>
<td>Pain medication can be administered while inside the multi-place chamber.</td>
<td>No evidence</td>
<td>No information available</td>
</tr>
<tr>
<td>Management of pain during treatment</td>
<td></td>
<td>Demonstrate reduction in pain scores</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Recommended therapeutic dose</th>
<th>HBOT (hyperbaric chamber)</th>
<th>Topical oxygen therapy</th>
<th>Topical oxygen perfusor / chamber</th>
</tr>
</thead>
<tbody>
<tr>
<td>How many treatments do I need?</td>
<td>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</td>
<td>Little information regarding generic dosage, length of time and use etc.</td>
<td>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</td>
</tr>
<tr>
<td>How often do I need them?</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Side effects</th>
<th>HBOT (hyperbaric chamber)</th>
<th>Topical oxygen therapy</th>
<th>Topical oxygen perfusor / chamber</th>
</tr>
</thead>
<tbody>
<tr>
<td>What I might experience</td>
<td>Visual changes—myopia (short sightedness) can occur after approximately 20 treatments. Vision usually returns to normal over time</td>
<td>No known detrimental effects to the wound bed</td>
<td>No side effects, reactions or allergies to product</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No side effects, reactions or allergies to product</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Probability of improvement</th>
<th>HBOT (hyperbaric chamber)</th>
<th>Topical oxygen therapy</th>
<th>Topical oxygen perfusor / chamber</th>
</tr>
</thead>
<tbody>
<tr>
<td>What can I expect with the process of healing</td>
<td>Does not immediately heal the wound HBOT provides highly oxygenated blood and creates a physiologically improved environment for healing</td>
<td>Limited evidence to healing potential. Promoted as supplying unobtrusive oxygen directly to the wound</td>
<td>Topical oxygen chamber: limited evidence of healing potential Topical oxygen perfusor: provide continuously pure oxygen to wound surface to stimulate wound healing</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Positive impact upon slough elimination and exudate reduction Granulox works to increase oxyhaemoglobin to the wound bed cells</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Changes in routine</th>
<th>HBOT (hyperbaric chamber)</th>
<th>Topical oxygen therapy</th>
<th>Topical oxygen perfusor / chamber</th>
</tr>
</thead>
<tbody>
<tr>
<td>How does this treatment affect my routine?</td>
<td>It is time consuming, may need to travel to the hyperbaric chamber and daily treatment will most likely take about 2 hours</td>
<td>Device has to be worn close to the body and may thus interrupt patients activities of daily living</td>
<td>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</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No change to patients daily routine. Patients can apply the product at their convenience</td>
<td></td>
</tr>
</tbody>
</table>
right modality and right time, with ‘due respect for any cultural, language and socioeconomic barriers’. O₂ therapy education is based on these essential components and allows the choice to commence O₂ therapy and which type/method of treatment/O₂ delivery is most suited to their situation to be made in a supported patient focused manner.

All O₂ 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 O₂ 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 O₂ treatment or O₂ enhancing product (haemoglobin spray).

In research undertaken in old ‘deck style’ multi-place, cylindrical hyperbaric chambers137,138 patients reported cold noisy air, feeling uncomfortable sitting, and felt only slightly reassured when they watched ‘desensitisation’ videos before treatment. Knight139 wrote of his personal experience that ‘treatment is dull’ while another study140 found

<table>
<thead>
<tr>
<th>Can I stop without disadvantage?</th>
<th>Yes—can cease HBOT or take a break. However break in treatment is discouraged, evidence supports continuity</th>
<th>There are no disadvantages to stopping the product suddenly</th>
<th>There are no disadvantages to stopping the product suddenly</th>
<th>There are no disadvantages to stopping the product suddenly</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complications</td>
<td>Patients with diabetes are likely to experience changes in blood glucose metabolism that will necessitate adjustment in diet and medication supervised by the doctor</td>
<td>Suitability of wearing device depending on location of wound</td>
<td>There are no considerations in regards to treatment safety</td>
<td>There are no considerations in regards to treatment safety</td>
</tr>
</tbody>
</table>

Table elaborated by Carol Baines and Sharon Hunt (Lead Advanced Nurse Practitioner, Independent specialist in wound care, Wellway Medical Group)
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 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, 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. 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 and Orsted provided evidence-based recommendations for practice and comment that the use of this therapy is well adopted by patients.

Several authors 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 O₂ 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 O₂ 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 O₂ treatment
- HRQoL of patients receiving O₂ treatment
- Advantages of O₂ therapy for the patient from their perspective.
- Exploration and expansion of research into health literacy associated with O₂ treatment. Research to explore the use of HBOT in the treatment of specific skin/wound conditions.
There is some direct evidence on the cost-effectiveness of HBOT in the treatment of acute and chronic wounds.\textsuperscript{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.\textsuperscript{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:

\textquote{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}'.\textsuperscript{141}

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.\textsuperscript{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.\textsuperscript{145} In Italy the economic indicators for using HBOT in DFUs showed potential saving of €19,000 per patient, which represents about 35\% savings.\textsuperscript{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.\textsuperscript{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.\textsuperscript{148}

The value of the HBOT for the money spent has been estimated in several countries considering the number needed to treat (NNT).\textsuperscript{149} In order to have a homogenous value for money spent, the cost of amputation was standardised for the NHS-\textsc{uk} value.\textsuperscript{150} The considered NNT for patients with DFUs is four for up to 35 HBOT sessions and three for more than 35 HBOT sessions.\textsuperscript{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.\textsuperscript{152}
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.

An RCT, which analysed costs in a group treated with O₂-releasing dressings compared with standard of care, failed to show significance. The mean cost per patient treated with the O₂ 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 O₂-releasing dressing. UK-based clinical studies have shown that, when added to standard care, haemoglobin spray could save the UK healthcare system an average of £2,330 for every patient with a DFU and £1,469 for every chronic wound patient after six months. Thus, there is an increasing clinical evidence that such adjunctive treatment has a positive impact on wound healing and cost reduction.

Fig 2. General considerations for use of oxygen therapies

<table>
<thead>
<tr>
<th>ODE</th>
<th>OWD</th>
<th>CDO</th>
<th>PDO</th>
<th>HBOT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxygen diffusion enhancer</td>
<td>Oxygen releasing wound dressing</td>
<td>Continuous delivery of oxygen</td>
<td>Pressurised delivery of oxygen</td>
<td>Hyperbaric oxygen therapy</td>
</tr>
<tr>
<td>Haemoglobin</td>
<td>Topical oxygen perfusors</td>
<td>Topical oxygen chambers</td>
<td>Hyperbaric chambers</td>
<td></td>
</tr>
</tbody>
</table>

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 O₂-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 O₂ is essential for healing of all kind of wounds. O₂ 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 O₂ in wound healing, the role of O₂ and hypoxia in the wound healing process, patient perspectives of these treatments, the cost-effectiveness of O₂ 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 O₂ 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 O₂ especially to the patient with non-healing wounds. This information may help the current planning and show the great potential for future treatment strategies.
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.


134 Sykes PK, FitzGerald M. Consumer engagement in the development of a video to inform health service clients about the risks and prevention of venous thromboembolism. European Journal for Person Centered Healthcare, 2015; 3(3).


Appendix A

GRADE recommendation explanation

The committee used the GRADE approach (Grades of Recommendation Assessment, Development and Evaluation) system\textsuperscript{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 between 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.\textsuperscript{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

<table>
<thead>
<tr>
<th>Recommendation</th>
<th>Benefit vs risk</th>
<th>Quality of evidence</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A</td>
<td>Clear</td>
<td>High: Consistent results from RCTs or observational studies with large effects</td>
<td>Strong recommendation, generalisable</td>
</tr>
<tr>
<td>1B</td>
<td>Clear</td>
<td>Moderate: RCTs with limitations and very strong observational studies</td>
<td>Strong recommendation; May change with further research</td>
</tr>
<tr>
<td>1C</td>
<td>Clear</td>
<td>Low: Observational studies Very Low: Case series, descriptive re-ports, expert opinion</td>
<td>Intermediate recommendation; Likely to change with further re-search</td>
</tr>
<tr>
<td>2A</td>
<td>Balanced or Unclear</td>
<td>High: Consistent results from RCTs or observational studies with large effects</td>
<td>Intermediate recommendation: May vary with patient values</td>
</tr>
<tr>
<td>2B</td>
<td>Balanced or Unclear</td>
<td>Moderate: RCTs with limitations and very strong observational studies</td>
<td>Weak recommendation; May vary with patient values</td>
</tr>
<tr>
<td>2C</td>
<td>Balanced or Unclear</td>
<td>Low: Observational studies Very Low: Case series, descriptive re-ports, expert opinion</td>
<td>Weak recommendation; Alternative treatments may be equally valid</td>
</tr>
</tbody>
</table>

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; 1795-187S.
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OXYGEN

“It is a fundamental clinical observation that wounds do not heal in tissue that does not bleed and they almost always heal in tissue bleeding extensively”. This statements comes from one of the most acknowledged oxygen researchers in the World, my old mentor Professor TK Hunt from San Francisco, USA.

The background for this statement is that continuous supply of oxygen to the tissue through microcirculation is vital for the healing process as well as resistance to infection. During wound healing the continuity and function of the damaged tissue is re-established by reconstruction of new vessels followed by new build up of connective tissue.

This is basic knowledge for all working clinically with wound patients in health care. This truth has, however, often been forgotten, when new developments have been presented in wound healing. I still remember when the focus some 20-25 years ago came on the importance of growth factors in the healing process. From experimental data it looks like all problems in wound healing were solved. However, in the clinical daily life these promising results were not found. One of the problems may have been that that in the excitement of the new advances, it was forgotten that the most basic process for cell survival is a constant delivery of oxygen. Even the best of the new advancements do not have any effect on cells or tissue lacking oxygen!

This issue of Journal of Wound Technology is for the mentioned reasons focusing on a compound, oxygen, which is of vital importance for basic process in wound healing. Different important topics are updated like pathophysiology, assessment tools and ways of deliver oxygen (topically and as hyperbaric oxygen).

The final indications for clinical use of oxygen in the wound area are still a major area of controversy. Like other areas in wound healing the practical use of oxygen has not yet been proven, if we look for the highest evidence level (I A) in the Cochrane System. More work consequently has to be done, before we know the optimal way to use oxygen in wound healing.

In spite of this, it is my hope that this issue of JWT will renew and improve the understanding of oxygen, and provide some practical information on oxygen assessment and delivery.

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WE VALUE YOUR OPINION!
We hope you enjoyed reading this issue of the Journal of Wound Technology. We are interested in your opinion and would be happy to receive your comments with a view to addressing our readers’ expectations. mbia@fr.oleane.com
Role of oxygen in wound healing and infection

Abstract

A continuous supply of oxygen is required for literally all aspects of wound healing and the resistance of wounds to infections, and evaluation of tissue perfusion and oxygenation is important in order to optimise the ability of the cardiovascular system to deliver an adequate volume of oxygen to meet the metabolic demands of repair. External administration of oxygen has been shown to significantly enhance both healing and immunity to wound infection. Hyperbaric oxygen therapy, though controversial, may be beneficial in situations where the nutritive flow and oxygen supply to the healing tissue are compromised and particularly if anaerobic infection is present. Oxygen in wounds depends heavily not only on the anatomic blood supply but on the activity of the sympathetic nervous system. Thus, external factors, smoking, cold, excessive pain, dehydration, certain medication and recreational drugs as well as the inspired oxygen level are important to healing and preventing and treating infection. This has created a need for improved methods of measuring oxygen in tissue, and a rationale for a strong relationship between anaesthesiologists, intensivists, and surgeons to optimize care for wounded patients.

Keywords: contamination, hyperbaric oxygen, hypoxia, supplementary oxygen, tissue perfusion, tissue oxygen tension

Introduction

Wound healing requires restoration of microcirculation to restore and replace injured vessels. The main, or at least the most "immediate" requirement is oxygen, which is critically important for reconstruction of new vessels and connective tissue and provision of a competent resistance against infection.

Oxygen at Cell Level

Wound healing involves recruitment of many enzymes, and many of the most important require oxygen as a substrate. The first event in wound healing is activation of an NADPH-linked oxidase. Within minutes it catalyzes the formation of superoxide, which is converted to hydrogen peroxide that then initiates chemattraction of leukocytes that is, itself, accelerated by increased oxygen. These events appear to prime leukocytes to ingest bacteria and tissue fragments etc. Phagocytosis activates another NADPH-linked oxidase called "nox" that quickly accelerates oxygen consumption for production of superoxide (O2-) and hydrogen peroxide that are injected into phagosomes where they initiate bacterial killing. This, for a while, dominates oxygen consumption, and PO2 in the wound falls quickly. The NADPH that is used in the reaction is regenerated by glycolysis leading to a large increase in glucose production of pyruvate and lactate that, in their turn, incite the development of angiogenesis factors, metaloproteinases, vascular endothelial growth factor and other wound-active substances. It is important to note that this production of lactate has nothing to do with hypoxia and remains high, even rises, when oxygen is increased. Thus, The NADPH-linked enzymes acting via ROS and lactate direct a major element of wound healing.

Collagen is among the genes that are activated by lactate/pyruvate. More oxygen is then required to hydroxylate collagen by the prolyl hydroxylase that transfers an oxygen atom to collagen that allows it to leave the cell and be cross linked by another oxygen consuming oxidase, lysyl oxidase, that adds to the development of tensile strength in the extracellular space. For instance, collagen deposition and development of strength is directly correlated to the partial pressure PO2 of the tissue (PPO2). Prolyl hydroxylases have a Km (concentration of substrate resulting in half the maximal rate of enzyme activity) of oxygen of about 25 mm Hg, and the production of collagen has been found proportional to PPO2. The most rapid production of collagen is theoretically reached at about 200 mmHg.

The Km of the NADPH-linked oxidases is about 50 mm Hg meaning that any reduction of oxygen in wounds impairs immunity and that increases to even 200 to 300 mm Hg, feasible in many cases, can raise immunity 2- to 5-fold.

When leukocytes make contact with the injured tissue, "nox," the acronym for neutrophil oxidase, is assembled and thereby activated as its 5 separate and inactive cytoplasmic parts are incorporated into the phagosomal membrane. A huge burst of oxygen consumption, as much as a 50-fold increase over basal follows, provided that enough oxygen is present. For a while this process is the largest oxygen consumer in the wound. The ROS perform a number of important functions, but at this time, this is the most notable.

Almost all of the superoxide/peroxide is channelled into the phagosome thus alkalizing it and pulling in K+ to balance the resulting charge disequilibrium and raising the redox potential. This appears to activate enzymes stored in the leukocytic granules to kill many types of bacteria, especially staphylococci and gram negative organisms.

Ubiquitination carries on from there by further marking
and disposing of the garbage. This, too, requires ATP and oxygen. If oxygen content is low, killing and ubiquination simply don’t happen, and infection becomes likely.

To go back a step, NADPH must be regenerated so that superoxide and lactate can be continued until all bacteria are killed. Glycolysis performs this function. Consequently, an excess of alpha hydroxy acids, that is, pyruvate builds up and with it, lactate. Note that this source of lactate has nothing to do with hypoxia, and, in fact is most likely increased by hyperoxia. Lactate dehydrogenase (LDH) is always present and maintains equilibrium of about 10 lactate to each pyruvate. Therefore, lactate increases, and with it begins the construction phase of healing by stimulating the transcription and post translational modification of wound related genes such as collagen gene, matrix metalloproteins, and others. This begins the destruction phase of healing and carries healing on to its proliferative phase and thus collagen deposition. The lactate also enhances TNF production by lipopolysaccharide, collagen lysis (remodelling), hif production and angiogenesis.

Thus, in short, the combination of lactate and increased oxygen is angiogenic, and productive of collagen tissue deposition as well as collagen lysis. This is counter-intuitive to most molecular biologists, but it is well defended. A so-called hypoxia-inducible factor, HIF has been identified as an important trigger for transcription of angiogenic factors. However, the idea that hypoxia is the stimulator is illusory! Instead, lactate reverses the action of a HIF prolyl hydroxylase that normally destroys HIF. Thus in wounds lactate increases the presence of HIF. Hyperoxia increases lactate (see above) that induces HIF and initiates a complex genetic cascade. This is opposite to the usual interpretation of the facts, but it is well defended by Lu and Varma. HIF-1 upregulates genes involved in glucose metabolism and angiogenesis under hypoxia or increased lactate, stimulates lactate production in what seems to be an amplification step to angiogenesis, and seems to protect cells from damage due, perhaps, to the oxidants that play such an important role in healing.

The production of epithelial tissue is primarily dependent on the degree of hydration and oxygen. While a moist wound environment increases the rate of epithelialisation by a factor 2-3, the optimal growth of epidermal cells is found at an oxygen concentration of 10-50%. Hyperbaric oxygen treatment increases the proliferation of the fibroblasts and the differentiation and epidermopoesis of the keratinocytes, but not the proliferation of keratinocytes.

Thus, oxygen is critical to literally all the components of healing including resistance to infection, and in every case, addition of oxygen increases the competence of healing. Delayed or stopped healing and development of infection are based on decreased perfusion, and subsequently oxygenation of the tissues. This is most clearly demonstrated by the extremely well-perfused, high \( P_{1O_2} \) tissue of the anal region, where the healing normally is excellent despite massive contamination.

### Oxygen at Tissue Level

\( P_{1O_2} \) is based on the following factors: 1. delivery of oxygen from the lungs to the tissue (oxygenation of arterial blood, circulation etc.); 2. Oxygen transport from blood to tissue (oxygen partial pressure in blood, the diffusion distance) and 3. Oxygen consumption in tissue. \( P_{1O_2} \) measurements in the wound tissue are by far the best way to observe the oxygen status of the tissue because it “reads” intracellular, extracellular, and blood in one number. Other methods, Doppler-based, infrared spectrophotometry, and haemoglobin saturation do not measure the \( P_{1O_2} \) that is the biochemically relevant number. The need for a device that can measure PO2 more conveniently in tissue currently retards advancement in the field. Electron spin resonance meets that need, but it is currently too unwieldy for clinical use.

Oxygen delivery is normally more dependent on oxygen bound to haemoglobin in the erythrocytes than of the arterial \( PO_2 \). This is true of muscle tissue that has small intercapillary distances and a high consumption of oxygen. In subcutaneous tissue, however, the intercapillary distances are higher and the consumption of oxygen is relatively low. Trauma of this tissue is followed by injured microcirculation and contraction of the vessels. Increased diffusion distances are increased and the partial pressure of oxygen \( (PO_2) \) becomes the major force for distribution of oxygen into the injured tissue. Slowly healing tissues as subcutis, tendon, fascia and bone then become dependent upon \( PO_2 \) in blood and tissue and to a lesser degree of the concentration of haemoglobin in blood. Anaemia with hematocrit values of 15-20% is normally of minor importance to the \( PO_2 \) in the wound and consequently of little import to healing. Subcutaneous tissue uses oxygen at a constant rate. One consequence of this is that a significant rise of \( P_{1O_2} \) in a wound after increased \( F_{1O_2} \) indicates adequate wound tissue perfusion, a useful trick to interpret the meaning of \( P_{1O_2} \) measurements.

Measurement of \( P_{1O_2} \) has been performed by introducing a small oxygen sensor in the tissue. Skin and subcutaneous tissue are first tissue to become hypoxic under sympthetic vasoconstriction due to blood volume deficits, cold, pain, etc. and the last to be normalised for which reasons this tissue is the optimal place for monitoring of general tissue perfusion.

In hyperbaric oxygen pure oxygen at a pressure of three atmospheres increases the diffusion distance of oxygen in the tissue by a factor 3-4 in the arterial end of the capillary and a factor two in the venous end. Hyperbaric oxygen treatment is limited by the time that it can be given, and at the usual frequency has shown little effect on the healing of normal uncomplicated wounds. However, there have been beneficial effects noted in complicated ischemic wounds in atherosclerotic or diabetic patients in whom wound \( PO_2 \) is very low. Recent RCTs have established the benefit of HBO in ischemic, infected diabetic foot ulcers (20-24) and a decreased risk of major amputation. Currently, such patients are being grouped according to assessment by transcutaneous \( PO_2 \) so as to eliminate patients who do not need more oxygen and those who do not respond to hyperbaric oxygen administration with an increase in \( PO_2 \).

### Influencing factors

Internal as well as external factors influence the \( P_{1O_2} \). In subcutaneous tissue the tissue perfusion is extremely dependent on haemodynamic conditions, cooling, pain, fear, smoking and medical compounds, particularly vasopressors and beta blockers. Many of these factors are found during surgery. Arterial hypoxaemia related to pain, opioids analgesics, and anaesthesia-induced atelectasis is frequently found the early postoperative hours, while the late hypoxaemia related to a decrease in lung capacity mainly based on a declined function of diaphragm is found 2-3 days postoperatively. Early hypoxaemia and reduced tissue perfusion enhance the risk of development of wound complications. The influence of late...
hypoxaemia on the contrary is not well studied, although it could have an adverse effect on healing.

Smoking is one of the most often debated external factors. In surgical patients smoking is known to increase the risk of necrosis of the wound edge, diminish cosmetic result, increased risk of wound infection in a variety of surgical ambulatory and in hospital surgical procedures, increasing risk anastomotic leakage after bowel surgery and increased recurrence rate after hernia surgery have been described.27,28 These damaging effects of smoking on the healing process are provided by different mechanisms.

Smoking one cigarette has been shown to decrease the tissue perfusion by more than 30% in more than 45 minutes in specific areas of the body.29 In such areas the production of collagen is 1.8 times higher in non-smokers compared to smokers (more than 20 cigarettes per day).30

The acute effects of smoking on wound oxygen may largely be due to nicotine. Nicotine is quickly absorbed, leading to a brief peak blood level, resulting in a peripheral vascular constriction followed by decrease in perfusion rate. Another contributor is CO in the cigarette smoke, which reduces the oxygen content of the blood. Nicotine patches did not result in wound healing defects30,31 and reason could be that they do not deliver the high initial nicotine blood level attained with inhaling cigarette smoke.30 Recent studies, however, have suggested that nicotine is not the major factor in smoking related diseases.

Smoking causes a temporary vasoconstrictive effect on blood flow and tissue oxygen, but a prolonged negative effect on collagen deposition, neutrophil oxidative killing mechanisms, growth factors, and metalloproteinases are affected. Recent evidence has demonstrated that nicotine does not affect the longer term mechanisms.33,34 The relative contribution of nicotine and other components of cigarette smoke are unclear, although the “poisoning” components have a longer effect and thus likely play a larger role.27

Smoking results in higher incidence of postoperative wound infections. This effect has even been described for minor, clean wounds.32 A significant difference in infection rate (12% in smokers compared to 2% in never-smokers; p=0.05) was found. Preoperative abstinence of smoking has been found significantly to reduce postoperative wound infections.26 Smoking cessation for a minimum of 4 weeks before surgery reduces postoperative wound infections, but is not enough to reverse the negative effect of smoking on tissue and wound dehiscence.34 Postoperative complications, recovery as well as long term health seem to benefit from smoking cessation lasting at least 4 weeks.35,36 Nicotine replacement therapy (NRT) is important because it increases the rate of smoking cessation by 50-70% and is largely independent of the intensity of support.27,28 Chantix, recently introduced, is also effective. The exact timing for abstinence to be of benefit, however, is unclear, and different benefits may require different duration of cessation. In Denmark 6 weeks of abstinence has been recommended before elective surgery.

Clinical Indications

Oxygen has for a long time been used in the clinic in order to enhance wound healing.

Locally oxygen has been applied to the wound surface in order to increase regeneration of epithelium. The effect of this treatment has been well documented but has been greeted sceptically largely because of the absence of randomized controlled trials.27 Systemic administration of oxygen through the lung and the cardiovascular system has been the preferred method for improved wound healing and decreased risk for surgical wound infection.35,37,38 Clinically, it has been shown that wound hypoxia is common in patients after major abdominal operations and that giving additional fluids significantly increases oxygen tension in the wound tissue and results in higher collagen deposition.40

Oxygen also has an important function in preventing surgical wound infection that remains the most frequent complication found in surgical wounds. As noted, bacteria in wounds are normally destroyed by intracellular oxidative mechanisms inside the leukocyte and molecular oxygen is necessary for production of superoxide that leads to innate oxidative killing. In animals, the oxygen concentration in the breathing mixture directly correlates to the size of the necrosis generated by dermal injection of bacteria.41 The critical level for this seems to be about 30-40 mmHg. In a human study of colorectal patients a direct correlation between subcutaneous PO2 and the resulting postoperative wound infection rate has been shown.42 If a rise of oxygen concentration in the breathing air did not result in an increased subcutaneous PO2, 45% of the patients developed a postoperative infection. If, however, the tissue perfusion was sufficient resulting in an increase of PO2 in subcutaneous tissue to 90 mmHg or more no patient developed a wound infection. Beside decreased production of oxygen radicals hypoxia causes a premature activation of the leukocytes resulting in a decreased effect on bacteria. Production of interleukin 2 and 8 is also decreased if hypoxia is present.43

In one third of all wound infections the bacteria found are sensitive to the prophylactic antibiotic that had been provided prior to incision.44 Decreased oxygenation may be the reason for this. Experimental studies have shown that antibiotics and oxygen are additive,45,46 and antibiotics are less effective in hypoxic wounds.47,48 While antibiotic delivery started more than 3 hours after the tissue trauma and bacterial contamination has no effect on the wound infection rate, oxygen has been shown to have an antibacterial effect even after 6 hours.49 Using the SENIC score system it was found that 40% of infections occurred in the 55% of patients classified as having uncontaminated wounds.44 Infection in clean wounds traditionally has been rationalised as due to unrecognised contamination. Reduced perfusion may be the reason for the decreased resistance for even small degrees of bacterial contamination.17

The use of supplementary oxygen in the inspired air has been for these reasons increasingly used clinically. However, this treatment, too, has also been a matter of debate. Clinical trials have reported conflicting results. In 2000, Greif et al.48 showed that colonic surgery patients benefited from as little as a few minutes preoperatively and two hours postoperative. An inspired oxygen concentration of 80% decreased the wound infection rate by half (11.2% against 5.2%; p=0.01) compared to an oxygen concentration of 30% oxygen administered during and 2 hours after surgery in combination with rigorously maintained normothermia, aggressive fluids and pain relief. Subsequent and similar clinical trials by Belda et al.49 reported a similar beneficial effect of perioperative supplemental high inspired oxygen in combination with aggressive fluids for reducing risk of surgical wound infections. Myles et al.50 showed that when nitrous oxide is removed and replaced in the breathing mixture by oxygen, wound infections diminished significantly. In contrast, Pryor et al.51 in a small population found that perioperative hyperoxia was not effective in reducing wound infections. However, the study was very poorly controlled. A
meta-analysis of the early results, a total of 3001 patients, came to the conclusion that perioperative administration of high inspired oxygen was effective. Unfortunately, these studies merely tell us that when fluids are restricted and temperature is not well controlled, the effect of oxygen cannot be found. Recently Meyhoff et al. reported the results of 1400 patients and found no significant difference between infection rates of SSIs in patients receiving 80% and 20% inspired oxygen. The authors of these studies did not measure P_{O2} and cannot, therefore conclude that raising P_{O2} in wounds has no effect. As with all of the dissenting studies, tissue P_{O2} was not measured, and as noted above, lack of effect cannot be inferred. Furthermore temperature was not rigorously controlled and fluids were restricted. However, they did prove that 80% oxygen given throughout the procedure caused no undesirable side effects.

Summary

What has been proved is that oxygen effectively prevents surgical wound infections but only when given simultaneously in combination with aggressive fluids and rigorously controlled normothermia. In the papers showing an effect of supplemental high-inspired oxygen there has been liberal fluid replacement, P_{O2} was significantly raised in the test group, and normothermia was maintained carefully.

Local hypoxia and bacterial contamination primarily are the responsibility of the surgeon, while the oxygenation of the patient is mainly based on anaesthesiological expertise. Therefore, an optimal collaboration between these groups is for this reason of vital importance. This is especially important for the organisation of oxygen treatment during surgery, in the recovery room and the first day postoperatively. Through development of combined standardised description of the treatment plan and a determined quality assurance of the patient course both for the pre-, per and postoperatively period the collaboration should be improved in the future. At this time, we still lack this consensus, and this paper is written to encourage it.

Development of more easily used devices to measure PO_{2} in tissue would hasten further advances in this field.

Conclusion

Adequate delivery of oxygen to injured tissue is vital for an optimal healing and resistance to infection. Evaluation of tissue perfusion and oxygenation and influencing external factors like smoking is important to optimise the hemodynamic condition and the ability of the cardiovascular system to deliver an adequate volume of oxygen. Although definitive proof of the effect oxygen therapy in clinical wound healing is established, the circumstances of its use are still debated.

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American Indians have believed for centuries their wounds would heal quicker if they hiked down into the "richer" air of the valleys.1 Modern hyperbaric wound therapy began in the 1960s, when famous oceanographer Jacques-Yves Cousteau built a village under the Mediterranean sea. In 1962, Conshelf 1 was set up off Marseille, France at a depth of ten meters. Cousteau and his team noticed that small scratches and wounds seemed to heal faster in the humid and oxygen-rich environment of the underwater houses. This discovery led to the development and proliferation of modern hyperbaric chambers and Hyperbaric Medicine.

Treating patients in hyperbaric chambers is costly and is associated with a number of risks. With that in mind, American neurosurgeon Boguslav H. Fischer began using a miniature version of a hyperbaric chamber that provided Oxygen topically to the wound.2 First results were published in 1966 and three years later The Lancet printed a report about 56 patients treated successfully with topical wound oxygen (TWO2).3 In the course of the next decades many scientists conducted research with topical oxygen system.22-28 In spite of very promising results, topical oxygen approaches remained in the shadows of more mainstream treatments.

Today a next generation TWO2 device is available in Europe providing enough reason for a critical appraisal of its biochemical mechanisms and clinical evidence of this new yet old concept.

Oxygen and Wound Healing

Oxygen \( \text{O}_2 \) is one of the major prerequisites for life. In mammals, all processes at the cellular level require \( \text{O}_2 \) which is provided in the majority via the adenosine triphosphate (ATP) pump. ATP cannot be stored and its synthesis requires \( \text{O}_2 \) and glucose. Interestingly the molecular mechanism and the ATP were only clarified in the 1980s. The scientists Paul D. Boyer and John E. Walker received the Nobel Prize in 1997 for their elucidation of the enzymatic mechanism underlying the synthesis of ATP. Most human organs receive their required \( \text{O}_2 \) via the circulatory and respiratory systems the largest human organ however is partly supplied with \( \text{O}_2 \) by diffusion directly from the ambient atmosphere. The border between external and internal supply seems to be the stratum corneum of the skin.29

A number of different factors play an important role in the development of chronic wounds. One of the most important is underlying disease associated with diminished perfusion and resultant reduced oxygen supply to the tissues. Among the most common are Diabetes Mellitus, arteriosclerosis and age. A wound requires \( \text{O}_2 \) to fight infection, to build up missing tissue and most other important processes in wound healing. In the wound healing cascade different cell types are important at different points of time, macrophages...
to fight infection, fibroblast for the synthesis of the extracellular matrix (ECM), collagen to fill the wound and epithelial cells to close the wound. All these cells need adequate O2 to fulfill their purpose. But O2 is not only the main source of energy.

In all phases of wound healing O2 is also needed as a substrate for essential enzymatic processes. In the first (Inflammatory) phase, neutrophils and macrophages build reactive oxygen species (ROS) which are important in fighting infection, intracellular and extracellular. When infected, the NADPH-linked oxidoase can increase the O2 consumption by as much as 50-fold. Up to 98% of the oxygen consumption of neutrophils is needed for ROS production. Newer research indicates that free O2 radicals are important for cell signaling to stimulate cell migration, cell proliferation and neo-vascularisation.

A means to describe the amount of O2 available is its partial pressure (pO2). While the normal pO2 in arterial blood is around 100mmHg, it is reduced to values around 40 at the wound edges and usually below 10mmHg at the center of chronic wounds. There are a number of reasons for low pO2.5 at the wound center. Trauma can destroy capillaries altering the diffusion distance for O2, Edema due to trauma or infection also increases the diffusion distance. As mentioned earlier, chronic wounds often are associated with age or diseases which are associated with limited blood flow. Simultaneously there is an increased need for O2 within the wound. High inflammatory activity, the need to build new ECM to fill the wound gap, the building of granulation tissue – all of these repair mechanisms need oxygen as a source of energy, as a substrate or signaling molecule.

It is worthwhile to have a more detailed look into the enzyme kinetics. The KM is the substrate concentration at which the reaction rate reaches half of its maximum value (Vmax/2). The concentration of O2 necessary to achieve half maximal ROS production (the KM) is in the range of 45–80 mmHg, with maximal ROS production at pO2 at >300 mmHg.36 As the pO2 in the center of the wound is regularly below a pO2 of 10 mmHg, the maximal effects of respiratory burst-dependent wound infection management can only be achieved through the administration of supplemental O2 to attain wound pO2 levels beyond those encountered when breathing room air.55 This also explains why the state of wound tissue oxygenation is a sensitive indicator for the risk of infection in surgical patients.24,27

Another important milestone in wound healing is the development of granulation tissue. Granulation tissue contains many capillaries and is of intense red color. Granulation tissue contains cells and extracellular Matrix (ECM). The ECM is built by fibroblasts and contains glycosaminoglycans, proteoglycans and collagen. Collagen is the main protein of the ECM and the human body. About 30% of the total proteins in humans is collagen. In the skin, collagen represents about 80% of the total protein mass. Consequently the production of collagen is essential for wound healing. Collagen synthesis requires O2 as a substrate in different enzymatic processes. Three peptide chains are hydroxylated in the endoplasmic reticulum to form a triple helical structure. This process is supported by the proline hydroxylase. After secretion outside the cell the lysyl oxidase needs O2 to form collagen fibrils via covalent cross-linking. This cross linking is essential for the stabilization of collagen fibrils and for the integrity and elasticity of elastin. When the function of the lysyl oxidase is reduced collagen is incomplete and less robust. Both Collagen and elastin are synthesized by fibroblasts. Endothelia cells need them in the building of vessels to stabilize the walls and keep the vessels elastic. Collagen synthesis is half maximal (KM) at a pO2 of 20–25 mmHg. Vmax is approximately 250 mmHg, suggesting that new vessels cannot even approach their greatest possible rate of growth unless the wound tissue pO2 is as high as 66.38 As the pO2 in the center of the wound is regular below a pO2 of 10 mmHg, hypoxic wounds deposit collagen poorly and are more likely to become infected.

Systemic hyperbaric therapy with pressures up to 2.5 atmospheres (2500mbar) enhances the arterial pO2 multiple but requires an intact capillary network to enhance the wound pO2. Consequently, local tissue oxygenation seems reasonable as no intact vasculature is needed. Unfortunately O2 has a very low solubility in watery environments. Therefore most experts believe that the topical application of oxygen would not be able to enhance the pO2 in the tissue.

Modern topical oxygen devices (like AOIT – Advanced Oxygen Therapy Inc.) address this problem with 2 components. First, highly concentrated O2 is administered directly onto the wound. Secondly, the devices work with a cycling pressure between 5 and 50mbar in order to further improve the diffusion gradient. The cycling pressure leads to a massaging compression without touching the wound.

In his first paper from 1966 Fisher reported that he didn’t achieve any healing results using devices with application pressures under 10mmHg.2 Therefore, the applied pressure seems to be extremely important in the topical application of O2. In 1975 Fisher measured the capillary pO2 in the finger tip as a comparison.4 The pO2 in the capillaries of the wound was less than 80mmHg at start and using a topical oxygen device with a pressure of 22mmHg the pO2 in the wound capillary was raised after one hour to 115mmHg and 120mmHg after two hours. The fingertip pO2 stayed constant at 96–97mmHg.

One year later Olejniczak also reported positive results in a study with 174 patients using a device using only 12mmHg.3 He measured the pO2 in granulation tissue near the wound surface and at a depth of 1 mm. pO2 in the plasma of the wound surface was raised from 50mmHg to 450mmHg and fell down to 50mmHg 2 minutes after stopping the O2 therapy. Olejniczak reported about great difficulties to measure the pO2 at 1mm depth back in 1976. He didn’t observe a raise of the pO2 during the therapy using 12mmHg pressure in the delivery device. When using nitrogen as a gas for the topical application the pO2 in the plasma of the wound surface fell from 50mmHg to 12mmHg after 5 minutes and stabilized later at 4.5mmHg. Since in this case any source of outside oxygen was eliminated the low values obtained represent an arterial supply of oxygen. This demonstrates...
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<td>Case study with 174 of various etiologies</td>
<td>Venous ulcers (102), arteriosclerotic ulcers (33), post surgical (33), sickle cell anaemia (4), lupus erythematosi (2)</td>
<td>Improvement in all wounds. 96% healing in venous wounds, 70% in ischemic ulcers</td>
</tr>
<tr>
<td>7</td>
<td>Diamond, 1982</td>
<td>The effect of Topical hyperbaric oxygen on lower extremity ulcerations</td>
<td>Case study</td>
<td>11 patients with wounds of various etiologies</td>
<td>Healing in “all cases”</td>
</tr>
<tr>
<td>8</td>
<td>Heng, 1983</td>
<td>Hyperbaric oxygen therapy for a foot ulcer in a patient with polyarteritis nodosa</td>
<td>Case study</td>
<td>1 patient with ulcer and polyarteritis nodosa</td>
<td>Healing</td>
</tr>
<tr>
<td>9</td>
<td>Heng, 1984</td>
<td>Hyperbaric oxygen therapy for pyoderma gangrenosum</td>
<td>Case study</td>
<td>2 patients with multiple ulcers on lower extremities and pyoderma gangrenosum</td>
<td>Healing in both cases after 6 and 12 weeks</td>
</tr>
<tr>
<td>10</td>
<td>Heng, 1984</td>
<td>A simplified hyperbaric oxygen technique for leg ulcers</td>
<td>Prospective, controlled study</td>
<td>Ischaemic wounds</td>
<td>5/6 patients in the $\text{TWO}_2$-group with 27 wounds healed 3 weeks vs. 0/5 in the control group</td>
</tr>
<tr>
<td>11</td>
<td>Ignacio, 1985</td>
<td>Topical oxygen therapy treatment of extensive leg and foot ulcers</td>
<td>Case study</td>
<td>15 patients of which 12 had diabetic ulcers, 12 osteomyelitis, 1 elephantiasis and 2 charcot feet</td>
<td>11/15 patients healed (73%)</td>
</tr>
<tr>
<td>12</td>
<td>Lehmann, 1985</td>
<td>Human Bite Infections of the Hand: Adjunct Treatment with Hyperbaric Oxygen</td>
<td>Semi-Randomized controlled study</td>
<td>43 patients with human bite wounds. 16 patients $\text{TWO}_2$ and 27 served as controls</td>
<td>Hospital stay was shortened from 4,7 days vs. 11,2 days in the control group</td>
</tr>
<tr>
<td>13</td>
<td>Upson, 1986</td>
<td>Topical hyperbaric oxygenation in the treatment of recalcitrant open wounds. A clinical report</td>
<td>Case study</td>
<td>2 patients with arterial ulcers</td>
<td>Both healed</td>
</tr>
<tr>
<td>14</td>
<td>Leslie, 1988</td>
<td>Randomized controlled trial of topical hyperbaric oxygen for treatment of diabetic foot ulcers</td>
<td>Prospective randomized study over 2 weeks</td>
<td>28 patients; 12 in $\text{TWO}_2$ group 16 controls</td>
<td>More than 55% reduction in both groups. No significant difference</td>
</tr>
<tr>
<td>NR.</td>
<td>AUTHOR/YEAR</td>
<td>TITLE</td>
<td>STUDY DESIGN</td>
<td>WOUND ETIOLOGY</td>
<td>RESULTS</td>
</tr>
<tr>
<td>-----</td>
<td>-------------</td>
<td>-------</td>
<td>--------------</td>
<td>----------------</td>
<td>---------</td>
</tr>
<tr>
<td>15</td>
<td>Landau, 1988</td>
<td>Topical Hyperbaric Oxygen and Low Energy Laser Therapy for the treatment of diabetic foot ulcers</td>
<td>Case study</td>
<td>50 patients with diabetic ulcers. 15 patients were only treated with TWO2 and 35 in combination of TWO2 and low energy laser</td>
<td>43/50 patients healed</td>
</tr>
<tr>
<td>16</td>
<td>Heng, 2000</td>
<td>Angiogenesis in necrotic ulcers treated with hyperbaric oxygen</td>
<td>Prospective, randomised study</td>
<td>40 patients with mainly pressure ulcers. Many of which associated with diabets and osteomyelitis</td>
<td>90% healed in the TWO2 group vs. 22% in the controls</td>
</tr>
<tr>
<td>17</td>
<td>Heng, 2000</td>
<td>Enhanced healing and cost-effectiveness of low-pressure oxygen therapy in healing necrotic wounds: A feasibility study of technology transfer.</td>
<td>Case study / virtual control group</td>
<td>15 patients with 24 wounds of different origin, 4 patients with osteomyelitis</td>
<td>22 out of 24 ulcers healed within 12 weeks. Significant cost reduction in the TWO2 treated patients</td>
</tr>
<tr>
<td>18</td>
<td>Landau, 2001</td>
<td>Topical Hyperbaric Oxygen and Low Energy Laser Therapy for Chronic Diabetic Foot Ulcers Resistant to Conventional Treatment</td>
<td>Case study</td>
<td>100 patients with diabetic ulcers treated with TWO and low energy laser</td>
<td>81% healed</td>
</tr>
<tr>
<td>19</td>
<td>Edsberg, 2002</td>
<td>Topical hyperbaric oxygen and electrical stimulation: exploring potential synergy</td>
<td>Case study</td>
<td>8 patients with pressure ulcers grade III and IV.</td>
<td>6/8 wounds healed within 16 weeks</td>
</tr>
<tr>
<td>20</td>
<td>Edsberg, 2002</td>
<td>Reducing epible using topical hyperbaric oxygen and electrical stimulation</td>
<td>Fallstudie</td>
<td>1 patient with grade IV pressure ulcer</td>
<td>Healed</td>
</tr>
<tr>
<td>21</td>
<td>Kallianen, 2003</td>
<td>Topical oxygen as an adjunct to wound healing: a clinical case series</td>
<td>Case study</td>
<td>58 wounds of various aetiology on 32 patients</td>
<td>65% healed without surgical intervention. 72,2% with surgical intervention (surgery/flap/graft)</td>
</tr>
<tr>
<td>22</td>
<td>Ishii, 2004</td>
<td>Efficacy of topical hyperbaric oxygen for refractory foot ulcer</td>
<td>Case study</td>
<td>2 patients with unspecified</td>
<td>Both wounds heal 3 and 9 month</td>
</tr>
<tr>
<td>23</td>
<td>Landau, 2006</td>
<td>Topical hyperbaric oxygen and low-energy laser for the treatment of chronic ulcers</td>
<td>Case study</td>
<td>274 patients; 218 patients with diabetic ulcer and 156 with venous ulcer</td>
<td>78% healing in both groups</td>
</tr>
<tr>
<td>24</td>
<td>Gordillo, 2008</td>
<td>Topical oxygen therapy induces vascular endothelial growth factor expression and improves closure of clinically presented chronic wounds.</td>
<td>Controlled study</td>
<td>57 patients; 32 HBO vs. 25 TWO2. Wounds of different etiologies</td>
<td>HBO did not reduce wound size. TWO2 reduced wound size and lead to higher VEGF</td>
</tr>
<tr>
<td>25</td>
<td>Tawfick, 2009</td>
<td>Does Topical Wound Oxygen (TWO2) Offer an Improved Outcome Over Conventional Compression Dressings (CCD) in the Management of Refractory Venous Ulcers (RVU)?</td>
<td>Controlled study</td>
<td>83 patients with venous ulcers. 44 patients with TWO2 and 37 controls receiving compression dressings</td>
<td>80% of TWO2 treated patients healed vs. 35% in the controls within 12 weeks</td>
</tr>
<tr>
<td>26</td>
<td>Aburto, 2010</td>
<td>A Randomized Controlled Trial to Evaluate Different Treatment Regimes with Topical Wound Oxygen (TWO2) on Chronic Wounds</td>
<td>Randomised, controlled study</td>
<td>20 diabetic ulcers and 20 venous ulcers. Every patient received TWO2 for 4 weeks. After randomization each 10 patients continued with TWO2 vs. advanced wound care in controls</td>
<td>Diabetic ulcers: 90% vs. 50% healing; Venous ulcers: 50% vs. 30% healed in 12 weeks</td>
</tr>
<tr>
<td>27</td>
<td>Blackman, 2010</td>
<td>Topical Wound Oxygen Therapy in the Treatment of Severe Diabetic Foot Ulcers: A Prospective Controlled Study</td>
<td>Controlled study</td>
<td>28 patients with diabetic ulcers. 17 received TWO2 and 11 advanced dressings</td>
<td>82% of TWO2 patients healed within 90 days vs. 43% in the controls</td>
</tr>
</tbody>
</table>
how important the atmospheric O\textsubscript{2} is for the supply of O\textsubscript{2} for the skin. Almost 30 years later Fries\textsuperscript{33} measured the diffusion of O\textsubscript{2} for a device using a higher pressure than Olleijniczak. He measured the pO\textsubscript{2} in pigs with artificial full thickness dermal wounds at a depth of 2mm with a device using 22mmHg of pressure. After 4 minutes of treatment the pO\textsubscript{2} in the center of the wound rose from values between 5-7mmHg to more than 40mmHg. Dual fluorescence staining of the tissue sections for smooth muscle actin and cell nuclei 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. Repeated treatment of the excisional dermal wounds in pigs clearly resulted in a significant acceleration of wound closure. Fries also showed that one of the most crucial vascular growth factors, VEGF was raised substantially in the topically treated wounds compared to the control wounds. These results were confirmed with humans by both, Scott and Gordillo who found enhanced VEGF concentrations after topical treatment with oxygen.\textsuperscript{25,34}

**Evidence of TWO\textsubscript{2} in wound healing**

We conducted a systematic literature review using the search string “topical oxygen” in PubMed. All publications were searched for secondary literature which were followed and obtained. As the number of Randomized Clinical Trials is limited, we abandoned a procedure usually used in health technology assessments that only look at RCTs. We don’t question the clear demand for well designed randomized clinical trials but also feel that a neglection of observational studies clearly limits innovation and new approaches.\textsuperscript{39} Table 1 summarizes the clinical publications. We limited this table to clinical studies. There are a number of publications that discuss the theoretical use of TWO\textsubscript{2} or review the available evidence. We are aware of a minimum of five position statements of different Hyperbaric societies. With the exception of the paper by Feldmeier in 2005,\textsuperscript{10} these position statements appear quite biased and seem to focus on supporting the reimbursement decisions in the countries where HBO is reimbursed as well as to discredit topical approaches. In this respect it seems to be useful information that for instance in the United States one session of hyperbaric treatment is reimbursed with up to 2,000 USD and up to 60 sessions.

Since the first study that we are aware of back in 1964, different authors and research groups have dealt with the subject of TWO and published more than 25 studies in the years thereafter. It is interesting to note that it took almost 50 years until a company developed a device that can be commercialized and is now available in most parts of the world. In summary there are more than 1,250 patients in studies published about TWO\textsubscript{2}. One weakness especially in the older publications is clearly that many studies did not clearly describe the population under investigation. Nevertheless more than 500 patients are clearly attributable to diabetic foot ulcers, almost 400 patients with venous ulcers and more than 120 to pressure ulcers.

The sheer number of patients is surprising. In the studies different devices and pressures where used. Some findings stick out. Clearly the applied pressure of the device seems important. Devices using less than 10mmHg seem to have little effect. Pressures around 22 mmHg appear to be clinically effective but may need a daily treatment duration of up to 12 hours. Only one device works with cycling pressures and provides humidified oxygen to prevent the wound from drying out. The cycling pressure reduces edema in a similar manner to compression dressings and shows good healing results with treatment times of 60 to 90 minutes.

**Summary**

In all phases of wound healing, oxygen plays a key role. Chronic wounds have a difficult challenge in that the need for oxygen is high while the supply of oxygen is low due to trauma, edema, limited vascularisation and underlying disease. Topical application of oxygen enhances the partial pressure of oxygen (pO\textsubscript{2}) to levels where various enzymes can effectively start healing. The effectiveness of Topical Wound Oxygen (TWO\textsubscript{2}) has been shown in a significant number of studies. However, there is a clear need for well designed randomized clinical trials to measure the true advantage of TWO\textsubscript{2} compared to other modalities like Hyperbaric Oxygen or advanced wound care. A new device is being commercialized that works with pressure gradients between 5 and 50mbar, showing excellent results with a clinically feasible treatment time of 60 to 90 minutes.

**References**


27. Aburto I, Frye C. A Randomized Controlled Trial to Evaluate Different Treatment Regimes with Topical Wound Oxygen (TWO2) on Chronic Wounds. Poster at Symposium of Advanced Wound Care (SAWC) and Wound Healing Society (WHS) Orlando, USA April 2010 (Accepted).


39. Black N. Why we need observational studies to evaluate the effectiveness of health care. BMJ. 1996 May 11; 312(7040):1215-8

# TOPICAL WOUND OXYGEN THERAPY (TWO2) AOTI Inc.

## DESCRIPTION / COMPOSITION
Topical Wound Oxygen Therapy product range includes both reusable and single-use systems that operate by applying cyclical oxygen pressure directly to the wound site within a sealed and humidified environment. This provides a greater tissue oxygen diffusion gradient and increased tissue oxygenation, which enhances antimicrobial actions, stimulates angiogenesis and maximizes collagen production. The cyclical nature of the pressure also creates a sequential compression effect which helps reduce peripheral edema and stimulates wound site perfusion.

## COUNTRIES WHERE THE PRODUCT IS AVAILABLE
USA, Canada, European Union, Russia, Middle East, Asia.

## UNDESIRABLE EFFECTS
None

## PRECAUTIONS
Oxygen rich environment precautions

## COST + COST EFFICACY
Results (>80% in 12 weeks) of complete wound healing of various ulcer types suggest excellent cost efficacy.

## PRESENTATION / DIMENSIONS
The Topical Wound Oxygen Therapy System is available with both reusable and single-use chamber options to meet your specific patient care and reimbursement needs.

## BIBLIOGRAPHY

## INDICATIONS
All acute and chronic wounds/ulcers, including:
- Diabetes ulcers
- Venous stasis ulcers
- Post surgical wounds
- Gangrenous lesions
- Decubitus/pressure ulcers
- Amputations/infected stumps
- Skin grafts
- Burns
- Frostbite

## CONTRAINDICATIONS
Acute deep vein thrombosis

---

**Reusable chamber**

**Single-use chamber**
**Topical Wound Oxygen Therapy (TW2) Single-use Sacral Unit AOTI Inc.**

**DESCRIPTION / COMPOSITION**

The Single-use Sacral Unit is designed to treat wounds on the torso, sacrum or hip. With an inbuilt adhesive ring the Sacral Unit is attached to the periwound area. It operates by applying constant oxygen pressure directly to the wound site within a sealed and humidified environment. This provides a greater tissue oxygen diffusion gradient and increased tissue oxygenation, which enhances antimicrobial actions especially against anaerobes, stimulates angiogenesis and maximizes collagen production.

**COUNTRIES WHERE THE PRODUCT IS AVAILABLE**

USA, Canada, European Union, Russia, Middle East, Asia.

**INDICATIONS**

All acute and chronic wounds/ulcers, including:
- Decubitus/pressure ulcers
- Post surgical wounds
- Gangrenous lesions
- Diabetic ulcers
- Venous ulcers
- Skin grafts
- Burns
- Frostbite

**PRESENTATION / DIMENSIONS**

The Single-use Sacral Unit comes in a case of 30.

**UNDESIRABLE EFFECTS**

None

**PRECAUTIONS**

Oxygen rich environment precautions.

**COST + COST EFFICACY**

Results of complete wound healing of various ulcer types suggest excellent efficacy and cost reduction. The Single-use Sacral Unit has demonstrated substantial effectiveness in infected wounds, including those colonized with MRSA.

**CONTRAINDICATIONS**

N/A

**BIBLIOGRAPHY**


Topical wound oxygen therapy (TWO) is widely used in North America and Europe. We initiated a clinical trial in 2010 to introduce this therapy into Japan; this involved six patients with chronic ulcers who underwent TWO. Pre- and post-treatment transcutaneous oxygen tension (TcPO2) values were evaluated at the periwound area. All cases showed increased TcPO2 values after TWO. In four cases, the size of the wound was reduced following treatment and there was formation of healthy granulation tissue. Wounds were completely closed by skin grafting in three of these four cases and healed spontaneously in one case. One of the cases is presented in detail here.

### Treatment protocol

Six patients with diabetic leg or foot ulcers ($n=5$) or sacral pressure ulcers ($n=1$) that had not healed in 3 months, despite use of best practice standard wound care — including surgical debridement and negative pressure wound therapy followed by moist wound dressings — were enrolled in the trial (Table 1). In addition to IRB approval from the Saitama Medical University Hospital, informed consent was obtained from each enrollee. Local topical wound oxygen therapy was applied along with best practice standard wound care. Sharp debridement was performed in five cases ($n=4$ diabetic ulcers; $n=1$ sacral pressure ulcer) to remove unproductive and infected tissue.

The single-use HyperBox topical wound oxygen (two2™) extremity chamber (AOTI, Oceanside, CA, USA) [Figure 1a] was employed for diabetic foot and leg ulcers, and the sacral topical hyperbaric oxygen chamber unit (AOTI) [Figure 1b] was employed for sacral pressure ulcers. Treatment was provided for 5 days a week, 90 minutes a day, according to the protocol recommended by the manufacturer. This treatment plan was continued for 4 weeks at the outset, or until spontaneous wound closure or sufficient granulation tissue formation was attained for operative wound closure via skin grafting.

The wound dressings were removed at the beginning of each treatment session. In the case of diabetic foot and leg ulcers, the affected
Figure 1. Topical wound oxygen therapy devices. a: Diabetic leg and foot ulcer extremity chamber device; and b. Sacral pressure ulcer unit device.

Figure 2. Oxygen tension before and after topical wound oxygen therapy. Pre- and post-treatment TcPO2 values surrounding the ulcers showed an increase in oxygen tension at 1 day after the initiation of therapy.

Table 1. Patient data

<table>
<thead>
<tr>
<th>Case number</th>
<th>Age</th>
<th>Sex</th>
<th>Wound site and size (including debrided area)</th>
<th>Complications</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>63</td>
<td>F</td>
<td>30 x 30 mm²: Right plantar</td>
<td>DM, HT, HL</td>
</tr>
<tr>
<td>2</td>
<td>70</td>
<td>F</td>
<td>100 x 40 mm²: Left toe necrosis</td>
<td>DM, PAD, CRF</td>
</tr>
<tr>
<td>3</td>
<td>55</td>
<td>M</td>
<td>40 x 30 mm²: Left 2nd and 3rd digit necrosis</td>
<td>DM, PAD</td>
</tr>
<tr>
<td>4</td>
<td>85</td>
<td>M</td>
<td>15 x 10 mm²: Left 5th digit necrosis and myelitis</td>
<td>DM, PAD</td>
</tr>
<tr>
<td>5</td>
<td>60</td>
<td>M</td>
<td>110 x 45 mm²: Right toe necrosis and myelitis</td>
<td>DM, PAD, CRF</td>
</tr>
<tr>
<td>6</td>
<td>80</td>
<td>F</td>
<td>100 x 120 mm²: Sacral pressure ulcer</td>
<td>DM, CRF</td>
</tr>
</tbody>
</table>

CRF: chronic renal failure, DM: diabetes mellitus, HT: hypertension, HL: hyperlipidemia, PAD: peripheral arterial disease.

The limb was placed into the inflated single-use extremity chamber. The integral cuff was then inflated to seal the limb within the chamber. On commencement of the treatment, the device delivered 100% oxygen into the chamber. The pressure then intermittently increased and decreased between 5 and 50 millibars (mb) [Figure 1a]. To prevent the wound from drying out during the treatment, humidification was provided by means of an ultrasonic humidifier. In the case of the sacral pressure ulcer, the hyperbaric oxygen chamber unit was placed over the wound site using an adhesive ring, with a bag placed over the oxygen delivery tube and a foam band placed around the torso to hold the unit in place [Figure 1b]. The oxygen supply tubing was then connected to an oxygen source, and the pressure in the unit was regulated to remain at 30 mb.

Generally, pressure ulcers are associated with bacterial infection or critical colonisation, which creates an extensive amount of exudate. Hence, these wounds do not normally require humidification to prevent drying. However, if required, humidification can be provided via a simple bubble jet device, as shown in Figure 1b. Consensus statements from an expert panel suggest that TcPO2 > 40 mmHg is usually associated with subsequent healing [15]. Transcutaneous oxygen tension (TcPO2) values surrounding the diabetic leg and foot ulcers and the sacral pressure ulcer were measured before and after treatment using a transcutaneous TCM400 oxygen monitor, as instructed by the manufacturer (Radiometer Medical, Copenhagen, Denmark). Wound size, wound recurrence and infection occurrence were assessed throughout the treatment period.

Outcomes

Six cases were examined in this evaluation. No complications were experienced by any of the six patients during topical wound oxygen therapy. The post-treatment TcPO2 in the vicinity of the ulcers were elevated at 1 day after treatment from the insufficient values (below 40 mmHg) to the adequate levels (above 40 mmHg) for wound healing in all six cases [Figure 2].

In four cases, robust tissue granulation was observed, and the wounds either healed spontaneously (n=1 diabetic ulcer) or were closed via skin grafting (n=2 diabetic ulcers; n=1 sacral pressure ulcer). One of these four cases (Case 3) is described in more detail below as a typical and successful case. Two additional diabetic ulcer cases (Case 4 and Case...
microbial growth inhibitory effect [3], and also by activating neutrophils [5]. Therefore, therapeutic strategies that improve the availability of oxygen to injured tissues are of great interest in the field of wound repair.

Japanese insurance only covers full body systemic hyperbaric oxygen therapy for chronic wounds, and there are many reports ascertaining the usefulness of this modality in wound healing. However, this therapy can only be performed in major hospitals, because the implementation of large-scale devices is required, in addition to the need for highly trained medical personnel with qualifications accredited by the Japanese Society of Hyperbaric and Undersea Medicine [16]. Furthermore, many contraindications are associated with the use of systemic hyperbaric oxygen therapy, which limits patient suitability, as do potential systemic complications, including neurotoxicity and alveolar damage [17,18].

In North America and Europe, alternative therapies are available that circumvent the risks and complications of systemic hyperbaric oxygen therapy by instead locally administering oxygen at the wound site. Topical wound oxygen therapy allows direct oxygen uptake by the injured tissue via an external delivery route, in contrast to full body systemic hyperbaric oxygen therapy, which relies on internal delivery via the vascular system. Moreover, topical wound oxygen systems are inexpensive and quite simple to use without the need for trained specialists. Topical wound oxygen therapy also does not pose the systemic risks seen with full body hyperbaric oxygen systems. Fischer first reported the usefulness of the new therapy in healing damaged tissue in 1969, even though the mechanisms of local oxygen therapy were unclear at that time [6]. Since then, elucidation of the advantages of direct oxygen uptake by the wounded tissue, and the development of topical wound oxygen devices, has resulted in enhanced interest and global use of this treatment.

Blackman et al [7] published a prospective controlled study in 2010 that explored the efficacy of topical oxygen therapy as an adjunctive modality in repairing diabetic ulcers that failed to heal by best practice standard wound care. All patients in the study received surgical debridement, offloading of the injured extremity, infection control, and selection of an appropriate dressing. The patients in the control group received silver-containing dressings, whereas the patients in the experimental group received simple dressings and local application of topical wound oxygen therapy.

Case 3
A 55-year-old man presented with a diabetic foot ulcer affecting the second and third digits. The ulcer is shown after surgical debridement [Figure 3a]. The TcPO2 value around the ulcer was very low, at 18 mmHg. Likely progression of necrosis was expected. However, a considerable amount of granulation tissue was formed at 4 weeks after the initiation of topical wound oxygen therapy [Figure 3b]. The wound healed spontaneously, and postoperative follow up indicated no ulcer recurrence in the following 2 months [Table 2].

Discussion
Numerous publications support the promotion of fibroblast and vascular endothelial cell proliferation, as well as collagen synthesis, by exogenous oxygen [1,2]. Oxygen also plays a major role in infection control [4] by providing a direct

**Table 2. Patient outcomes**

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Ischaemia</th>
<th>Revascularisation</th>
<th>Clinical outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>No</td>
<td>No</td>
<td>Healed by skin graft, no recurrence during 12 months</td>
</tr>
<tr>
<td>2</td>
<td>Yes</td>
<td>No</td>
<td>Healed by skin graft, no recurrence during 4 months</td>
</tr>
<tr>
<td>3</td>
<td>Yes</td>
<td>No</td>
<td>Healed spontaneously, no recurrence during 2 months</td>
</tr>
<tr>
<td>4</td>
<td>Yes</td>
<td>Yes</td>
<td>Self-discharged</td>
</tr>
<tr>
<td>5</td>
<td>Yes</td>
<td>Yes</td>
<td>Lower limb amputation</td>
</tr>
<tr>
<td>6</td>
<td>No</td>
<td>No</td>
<td>Healed by skin graft, no recurrence during 8 months</td>
</tr>
</tbody>
</table>

Figure 3. Diabetic foot ulcer; a. The debrided wound is shown prior to commencing topical wound oxygen therapy. b. The wound showed formation of healthy granulation tissue at 4 weeks after the commencement of oxygen therapy.
of oxygen for 60 minutes, 5 days a week. The complete healing rate after 12 weeks of topical wound oxygen therapy was an impressive 82.4% in the experimental group versus only 45.5% in the control group. Furthermore, the mean time to complete healing was significantly reduced in the experimental group compared with the control group (56 versus 93 days). The patients in the treatment group showed very low recurrence rates after 18 months, which was likely related to the augmented patency of the interlaced collagen fibers produced in the high-oxygen environment.

Tawfick and Sultan also investigated topical wound oxygen therapy in a prospective controlled study involving 83 patients with refractory venous ulcers. Both the control and the experimental group received best practice standard wound care from a team of vascular surgeons at a university hospital. Wound care included compression therapy in the control group, and daily local oxygen therapy in the experimental group. After 12 weeks of treatment, 80% of the ulcers were completely healed in the oxygen therapy group, as opposed to 35% in the compression therapy group. Similar to the Blackman et al. study, the mean time to complete healing was significantly reduced in the experimental group relative to the control group (45 versus 182 days). The patients were followed up for 36 months. The 2013 follow-up report demonstrated recurrence in 14 of the 30 healed ulcers in the compression therapy group, compared with only three of the 51 in the oxygen therapy group.

The purpose of the current study was two-fold:

- To investigate the effect of topical oxygen therapy during the early stages of wound healing (as assessed by the formation of healthy granulation tissue, or immediately after surgical debridement but before skin grafting)
- To investigate the effect of this therapy on infection control.

TcPO2 values were measured around the ulcer before and after topical wound oxygen therapy and, in all cases, the TcPO2 was elevated after treatment. Four of the six patients presented with considerable formation of healthy granulation tissue and a decreased wound area. In the two patients in whom a significant therapeutic effect was not obtained (Cases 4 and 5), the TcPO2 values before treatment were extremely low (<10mmHg). Hence, topical wound oxygen therapy may not be wholly curative in extremely severe ulceration cases. Additional studies with a larger number of patients are essential to validate long-term results, and to establish adaptation criteria.

**Conclusions**

These case reports showed that local administration of topical oxygen to chronic diabetic foot and leg ulcers and to a sacral pressure ulcer effectively increased the TcPO2 values in the periwound area. Topical wound oxygen therapy required no special skills, lending itself to ready application under most circumstances, even at a home site. The treatment has an extremely low risk of systemic complications, and single-use devices greatly reduce the possibility of secondary infections. Therefore, this adjunctive treatment modality is considered a useful means of treating chronic ulcers together with best practice standard wound care. Following this clinical study the authors are now trying to carry forward the procedures to obtain the approval of the device from the Pharmaceuticals and Medical Devices Agency of Japan, as well as resultant reimbursement by the Japanese national insurance system.

**Conflict of interest:** The topical wound oxygen therapy devices used in the study were provided by AOTI, Oceanside, CA, USA. The authors have no commercial, proprietary, or financial interest in the devices or the manufacturing company.

**References**

A merican Indians have believed for centuries that their wounds would heal quicker if they hiked down into the ‘richer’ air of the valleys[1]. Modern hyperbaric wound therapy began in the 1960s, when famous oceanographer Jacques-Yves Cousteau built a village under the Mediterranean sea. In 1962, Conshelf[2] was set up off the coast of Marseille, France at a depth of ten metres. Cousteau and his team noticed that small scratches and wounds seemed to heal faster in the humid and oxygen-rich environment of the underwater houses. This discovery led to the development and proliferation of modern hyperbaric chambers and hyperbaric medicine.

Treating patients in hyperbaric chambers is costly and is associated with a number of risks. With that in mind, American neurosurgeon Boguslav H. Fischer began using a miniature version of a hyperbaric chamber that provided oxygen topically onto the wound[3]. First results were published in 1966 and three years later, The Lancet printed a report on 56 patients treated successfully with topical wound oxygen[4].

Oxygen is one of the major prerequisites for life. In mammals, all processes at the cellular level require oxygen, which is chiefly provided via the adenosine triphosphate (ATP) pump. ATP cannot be stored and its synthesis requires oxygen and glucose. Interestingly the molecular mechanism and the ATP pump were only clarified in the 1980s. The scientist Paul D. Boyer and John E. Walker received the Nobel Prize in 1997 for their elucidation of the enzymatic mechanism underlying the synthesis of ATP. Most human organs receive required oxygen via the blood circulation and the lungs. However, the largest human organ — the skin — is partly supplied with oxygen by diffusion directly with the atmosphere[5]. The border between external and internal supply seems to be the stratum corneum of the skin.

In all phases of wound healing oxygen is also needed as a substrate for essential enzymatic processes. In the first (inflammatory) phase, neutrophils and macrophages build reactive oxygen species (ROS) which are important in fighting infection. When infected, the NADPH-linked oxidase (nicotinamide adenine dinucleotide phosphate-oxidase, a membrane-bound enzyme complex) can increase oxygen consumption by as much as 50-fold. Up to 98% of the oxygen consumption of neutrophils is needed for ROS production. Newer research indicates that free oxygen radicals are important for cell signaling to stimulate cell migration, cell proliferation and neovascularisation[6].

Oxygen delivery is a critical element in the healing of wounds. The pathophysiology of lack of oxygen in wounds is known with a high evidence level. However, there is a lower level of clinical evidence, which may lead to a lack of topical oxygen use in wound care. Further clinical research in this area is therefore needed, so this case study by Hitomi and Shigeru is welcomed. [WINT]

References

Author: Tom Wild, General Surgeon; Wound Center Dessau-Rosslau, Department of Dermatology, Dessau Municipal Hospital Dessau, Germany
Measurement of Tissue Oxygenation with a Hyperspectral Tissue Camera System before and after Topical Wound Oxygen (TWO₂) therapy

Male Patient, 82 years old, Peripheral Artery Disease (PAD)

<table>
<thead>
<tr>
<th>Main diagnosis</th>
<th>Secondary diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Severe Arterial ulcer due to PAD of lower right leg</td>
<td>Chronic venous insufficiency</td>
</tr>
<tr>
<td>Fontaine Stage IV: Necrosis / gangrene of the limb</td>
<td>Coronary heart disease with bypass surgery in 1986 and 1994</td>
</tr>
<tr>
<td>Condition remains after Percutaneous transluminal angioplasty (PTA) of A. tibular. post., A. fibular, A. plantar right (08/2014)</td>
<td>Arterial hypertension. Intermittent atrial fibrillation</td>
</tr>
<tr>
<td>Condition remains after PTA of A. tibular anterior, A. fibular right (03/2015)</td>
<td>Prostatectomy for prostate carcinoma in 2006</td>
</tr>
<tr>
<td></td>
<td>Right total hip replacement in 2007</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Beginning of 2016</th>
<th>August 2015 - September 2016</th>
</tr>
</thead>
<tbody>
<tr>
<td>New formation of a deep ulcer in the area of the back of the right foot with transition to lateral foot</td>
<td>Surgical treatment of the extensive ulceration with Reverdin Skin Graft fails and ulcer size increases</td>
</tr>
</tbody>
</table>

Hyperspectral Camera measurements made Before and After the application of Topical Wound Oxygen (TWO₂) therapy for 60 mins. Comparison of the Before and After TWO₂ application

Hyperspectral measured values:

1. Significant improvement of oxygen saturation in the superficial tissue (StO₂)
2. Significant improvement of oxygen saturation in the deeper tissue layers (NIR)
3. Increase of total hemoglobin (THI)
4. Partially lasting effects in the entire distal lower limb and foot area
About TIVITA™ Tissue – The Hyperspectral Tissue Camera for Objective Measurements of Physiological Parameters

The TIVITA™ Tissue (Diaspective Vision GmbH, Germany) is an innovative imaging technology for the easy and flexible assessment and documentation of tissue areas. It allows for the contact-free, non-invasive recording of various tissue parameters of surfaces over larger areas. Important parameters such as Tissue Oxygenation and Perfusion can be measured within a few seconds.

The TIVITA™ Tissue System uses the Chemical Color Imaging Technology for data acquisition, calculation and display.

The visible (VIS) and near infrared (NIR) spectral range is recorded by the image acquisition system. It is like an imaging tissue oximeter and relies on the latest advantages in hyperspectral imaging. Instead of a single-spot-reading, the system creates several pseudo-color images of each parameter. For the measuring procedure, the TIVITA™ Tissue is placed approx. 50 cm above the subject.

About Advanced Oxygen Therapy (AOTI) and Topical Wound Oxygen (TWO₂) therapy

AOTI is dedicated to providing Advanced Wound Care products that utilize our patented non-invasive TWO₂ therapy. Our innovative products can help close all wound types, and are particularly effective in chronic (diabetic, pressure, and venous ulcers) and acute (post-surgical, cosmetic, and burn) wounds.

TWO₂ therapy is unique in that it can be applied at the patient’s home or in any healthcare setting and helps to address the fundamental reasons chronic wounds fail to heal, by; reversing wound tissue hypoxia, stimulating the underlying cellular mechanisms needed for tissue regeneration, creating non-contact sequential compression to reduce edema and promote perfusion, and creating an environment that destroys unwanted pathogens and combats infection.
A Randomized Controlled Trial to Evaluate Different Treatment Regimes with Topical Wound Oxygen (TWO2) on Chronic Wounds

Aburto I1, Frye C2
1 Instituto Nacional de Heridas (INH), Santiago, Chile, 2 AOTI Ltd., Galway, Ireland

Introduction
Chronic wounds on the lower leg and foot are frequent, difficult to treat and show high rates of complications1). After very positive results with a unique pressurized topical oxygen therapy (TWO2) device in other studies2,3) we investigated whether 4 weeks of TWO2 treatment and consecutive 8 weeks of advanced moist wound treatment (AMWT) is equally effective in healing chronic wounds as continuous treatment with TWO2.

Method
The randomized, controlled study was conducted at the National Wound Institute in Santiago de Chile. In an outpatient setting with patients with severe diabetic foot ulcers (DFU) (n=20) and chronic venous ulcers (CVU) (n=20) all patients received TWO2 for a period of one month. Then the groups were randomized to continue with TWO2 (TWO2-TWO2 group) or receive AMWT for 2 more month (TWO2-AMWT group). TWO2 patients were treated daily for 2 hours 5 times a week. The device delivered humidified medical grade oxygen with pressure cycles between 5 and 50 mbar. Dressing changes in the control group were performed according to best practice at a minimum of twice a week. The primary endpoint was complete ulcer closure after 90 days.

Results
The majority (82%) of the patients were referred to the study center for minor or major amputation. All of these patients improved under the therapy and no patient underwent amputation. Patients were comparable concerning age, size of the wound and duration of the wound. 90% of the DFU patients in the TWO2-TWO2 group healed within 90 days vs. 40% in the TWO2-AMWT group. Patients with CVU had 50% healing vs. 30%, respectively.

Conclusion
Patients with complicated ulcers benefit from the treatment of topical localized oxygen (TWO2). Continuous TWO2 treatments for 12 weeks showed significant better outcomes than a shorter TWO2 treatment regime of 4 weeks followed by AMWT.


Presented at EWMA 2010 May 26-28, Geneva Switzerland
Topical Oxygen Treatment (TWO₂) in Two Cases With Pressure Ulcers in Finland

Aino Kivelä, Nurse, HUS; Helsinki University Hospital, Toolo

Introduction

In spring 2009 I tried the Topical Wound Oxygen TWO₂ therapy manufactured by AOTI Ltd, Ireland, with two patients with spinal cord injuries caused by an accident. For the treatment I used the sacral patches designed for wounds at the trunk of the body. This system delivers humidified oxygen at a continuous pressure of 30 mbar to the wound bed. The required oxygen was obtained by a SeQual Oxygen CE-Marked for wound care. The course of treatment was 1 hour per day.

Case 1:

A 26 year-old female patient with an entire spinal cord injury caused by a car accident. On the sacrum, above the cross bone, there was a II grade pressure (EPUAP) ulcer of size of 1.5 cm x 1.5 cm. The healing of the ulcer was stalled despite many different approaches of treatment. TWO₂ therapy was given once per 24 hours with duration of one hour. During the treatment the patient was in bed lying on his side. After the treatment the wound was of scarlet colour and “bloodish”. After nine days of treatment the maceration was vanished and the uneven/rough edges of the wound were tidy. The TWO₂™ therapy was administered further to support the standard local treatment. The wound showed good granulation tissue after a few days. TWO₂ was continued for a period one month. During this time the wound did not close but showed very good granulation tissue as well as reduction in wound size and depth.

Case 2:

A male patient with a partial spinal cord injury after being run over by a train. In the lower back was a re-opened post surgical wound that probably developed due to pressure. After starting TWO₂ the wound healed drastically quicker compared to the previously used treatment. Within 3 weeks the wound was closed.

Conclusion

TWO₂ seems to enhanced granulation, cleaning and healing of pressure ulcers. Administering the therapy does not require any skilled medical personal, but a trained wound care nurse should follow up the healing process.
The Use of Topical Wound Oxygen (TWO2) in a Complicated Acute Venous Embolism and Thrombosis of the Lower Extremity

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

Conclusion: TWO2 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.

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 TWO2 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 TWO2 Treatments 90 min/BID

Anticoagulation: 7.5 mg Warfarin x 30 days

S/P 10 weeks TWO2 Treatments 90 min/BID

Anticoagulation: 5.0 mg Warfarin x 30 days

S/P 16 weeks TWO2 Treatments 90 min/BID

Fem-Pop bypass: patent
The Use of Topical Wound Oxygen (TWO2) in a Complicated Post Surgical Transmetatarsal Amputation with Incision and Drainage of the Foot

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

A 47 yr/o Female with a hx of severe DM, Retinopathy, and Neuropathy presented to the Emergency Department with a severe left foot infection. The patient presented very confused and had not seen a provider in over a year. She stated the ulcer started as a blister on the bottom of her foot and was receiving care by her immediate family.

A multidisciplinary team approach was attained and collaboration was established with Medicine, Vascular Surgery, and Infectious Disease. The patient had palpable pulses (2/4) and were audible upon bedside testing. The patient presented with a 560 glucose level along with normocytic anemia with an H/H of 7.9/25.3. Two units of packed RBCs were given during surgery and 2 more units were given at post op day 1. The patient had a spike in her WBC at post op day 1 which was attributed to the transfusion. A negative pressure device was used for 3 days and then discontinued due to pain and discomfort. Topical Wound O2 therapy was initiated following surgery bid for 90 mins.

The patient was discharged on post op day 6 and was placed on po Augmentin 500/125 mgs bid for 14 days. Wound dressings consisted of light wet to dry packing changed bid in conjunction with TWO2 therapy bid/90 mins. The patient was placed in a removable posterior splint for 3 weeks and then transitioned to a CAM boot until healed. Once healed, the patient was placed into a custom molded shoe with filler.

Conclusion: This is a very complicated case of a Diabetic Foot infection that responded favorably to a multidisciplinary approach and Topical Wound O2 Therapy. The TWO2 was very effective not only from a wound healing perspective, but also in providing the patient with comfort, direct involvement with her wound care, and ease of use at home.
The Use of a Mesenchymal Stem Cell Living Skin Substitute in Conjunction with Topical Wound Oxygen for an Ischemic Post Operative Transmetatarsal Amputation

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

A 66 yr/o Male with a hx of severe PVD, CVA, CHF, Hep C, s/p BKA, End Stage Renal Dx, and DM Underwent a TMA of the Left foot secondary to Osteomyelitis and infection. Immediately post operatively, the wound became escharotic and dehisced. The patient was then placed on Topical Wound Oxygen Therapy (TWO2) for wound staging and wound bed preparation.

The patient underwent a Vascular Bypass Graft 5 months prior to the TMA procedure. Pre and Post NIVs were N/C. The patient was not a candidate for further vascular surgery and presented with a natural hx of limb loss on the contralateral side prior secondary to PVD and infection.

ABIs: Left Not compressible (N/C)
TBIs: (TMA)
S/P: SFA-PTA Bypass
Non palpable pulses
Dopplers: non audible
Waveforms: flat line

Hx of Smoking: 1 PPD / 35 yr Pack hx

Left TMA: Immediately Post Op
Grafix Core Preparation
Application of the Grafix Core Mesenchymal Stem Cell Living Skin Substitute within the dehisced wound. Wound Measurement 7.5 x 2.8 cm
Application of Steri Strips
S/P 2 applications of Grafix Core (4weeks)
The Patient continued with TWO2 until full healing.

S/P 5 Weeks TWO2 Therapy 45 min/BID

Time of Healing | X-rays | Blood Cultures | Lab Analysis
--- | --- | --- | ---
9 weeks | negative | negative | WBC/CRP/SED rate

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Topical Wound Oxygen (TWO2) used with Standard Best Practice Wound Care on Recalcitrant Lower Extremity Ulcers

Christopher Japour, DPM VAMC, Northport, NY • Edward Chen, DPM, MDVAMC, Danville, IL • Praveen Vohra, DPM, Plainfield, IL

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 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 (1 month-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 a way 3 months later from an acute MI.

Patient 2 - MEDICAL PATIENT WITH TX 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 x 1.3 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 presents to the clinic with a past medical history of ostearthritis, 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 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 dehiscended 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, ostearthritis, 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-ulcer 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 dehiscended 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.

Graph 1: Data tables for patients and ulcer characteristics.
The Use of a Human Fibroblast-derived Dermal Substitute with Topical Oxygen in Vascular Compromised Wounds

Dr. Francis Derk - South Texas Veterans Health Care System: Chief of Podiatry, University of Texas Health Science System: Adjunct Clinical Staff
Commander: United States Navy, 4th Medical Battalion - Officer-in-charge H&S Company, Det 2 PHX, AZ.

Introduction
Wound care in compromised patients with insufficient blood flow and that are not candidates for by-pass offers a unique challenge for treatment. These patients are excluded from clinical trials yet they pose to be some of the most difficult to treat. Studies have demonstrated the cost to treat chronic wounds can range from $13K in uncomplicated ulcers to over $80K in complicated ulcers. More importantly is the high mortality and unilateral amputation rate associated with chronic wound patients.

Purpose
To evaluate the efficacy and speed of closure using patients received weekly applications of a Human Fibroblast-derived Dermal Substitute, topical oxygen and conventional wound care consisting of infection control, debridement, off-loading or compression.

Methods
We evaluated 12 ulcers on 9 patients; 2 venous stasis, 5 post operative dehiscences, and 5 DFU’s. All patients received weekly applications of Human Fibroblast-derived Dermal Substitute, topical oxygen and conventional wound care consisting of infection control, debridement, off-loading or compression. Average ulcer size was 4.6 X 1.9 cm. One patient also had a sinus tract. All patients had significant PVD, Renal Disease and 6/9 were chronic smokers. All patients were not candidates for by-pass surgery. 6/9 patients were non-compressible and the other three patients ABIs were less than .7. The average TBI on the 9 patients ranged from .1-.7 with an average of .33. The average age of our patients was 70 years old (53-81). All patients were unresponsive to conventional wound care, and negative pressure.

Results
9/9 patients who were not candidates for by-pass achieved closure with the combination of weekly applications of a Human Fibroblast-derived Dermal Substitute, topical oxygen and conventional wound care consisting of infection control, debridement, off-loading or compression. Average time to closure was 12.7 weeks.

Conclusion
Multi-modality wound care to close wounds faster and in patients that fail to heal with single modality offers a therapeutic benefit for patients that haven’t responded to therapy in the past. Additionally, if we can close wounds faster, especially in an extremely at risk population, we will reduce the complications associated with chronic wounds with and lower the overall treatment cost for our Veterans.
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 HBO2 or TWO2 modalities. Topical wound care and off loading consisted of wet to dry dressings and Cam boots respectively. The TWO2 patient lived over 65 miles from the nearest HBO2 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.

<table>
<thead>
<tr>
<th>Patient Data</th>
<th>HBO2</th>
<th>TWO2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>52</td>
<td>64</td>
</tr>
<tr>
<td>DM</td>
<td>13 yrs</td>
<td>21 yrs</td>
</tr>
<tr>
<td>Hx of amputation</td>
<td>x2</td>
<td>x1</td>
</tr>
<tr>
<td>PMH</td>
<td>DM, HTN, Hep C, Cirrhosis</td>
<td>DM, HTN, Obesity, Kidney Dx</td>
</tr>
<tr>
<td>Smoking</td>
<td>20 pack yrs</td>
<td>Negative</td>
</tr>
<tr>
<td>ABI/TBI</td>
<td>.85 / .45</td>
<td>.90 / .52</td>
</tr>
</tbody>
</table>

Results:

- The TWO2 wound took 17 days longer to heal
- TWO2 costs were less expensive
- TWO2 was utilized to full closure vs HBO2 which was limited to 40 dives (day 56 and not fully healed)
- The HBO2 wound apart from 40 dives required 32 additional days of conventional wound healing to closure
- No baro-trauma or complications were incurred

HBO2 and TWO2 are both viable options in healing large open wounds. TWO2 has been shown to be cost effective, and a comparative healing modality. TWO2 is an excellent, alternative choice to HBO2 especially when considering financial resources, limitations with health, availability, and convenience (home usage).
Treatment of a Chronic Stage IV Pressure Ulcer using Topical Wound Oxygen (TWO2) Therapy

Anku, Comfort RN, Dr. Christian Frye2
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 (TWO2) 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 TWO2 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 TWO2 treatment, the wound was treated with Silversorb and Betadine then redressed with standard gauze dressing. The TWO2 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. TWO2 therapy was discontinued during the hospitalization. TWO2 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. TWO2 improves local tissue perfusion
2. TWO2 softens necrotic tissue and enhances debridement
3. TWO2 eliminates maceration
4. TWO2 reduces nursing intervention time

Conclusion
Patients with severe chronic wounds benefit from the treatment with TWO2 and show remarkable wound closure rates.
The use of Topical Wound Oxygen and Human Fibroblast-derived Dermal Substitute in Vascular Compromised Wounds

Dr. Francis Derk, CDR, USN
South Texas Veterans Health Care System: Chief of Podiatry

Introduction
Wound care in compromised patients with insufficient blood flow and who are not candidates for bypass offers a unique challenge for treatment. These patients are excluded from clinical trials yet they pose to be some of the most difficult to treat. Studies have demonstrated the cost to treat chronic wounds can range from $13K in uncomplicated ulcers to over $80K in complicated ulcers. More important is the high mortality and unilateral amputation rate associated with chronic wound patients.

*Average cost per Ulcer Episode

$8,000 → Uncomplicated Wound

$45,000 → Amputation

Purpose
To evaluate the efficacy and speed of wound closure on patients receiving Topical Wound Oxygen, weekly applications of Human Fibroblast-derived Dermal Substitute (HFDS) and conventional wound care consisting of infection control, debridement, off-loading or compression.

Method
We evaluated 12 ulcers on 9 patients; 2 venous stasis, 5 post operative dehiscences, and 5 DFU’s. All patients received Topical Wound Oxygen, weekly applications of Human Fibroblast-derived Dermal Substitute, and conventional wound care consisting of infection control, debridement, off-loading or compression therapy. Average ulcer size was 4.6 X 1.9 cm. One patient also had a sinus tract. All patients had significant PVD, Renal Disease and 6/9 were chronic smokers. None of the patients were candidates for bypass surgery. 6/9 patients were non-compressible and the other three patients’ ABIs were less than .7. The average TBI on the 9 patients ranged from .1-.7 with an average of .33. The average age of our patients was 70 years old (53-81). All patients were unresponsive to conventional wound care and NPWT.

Results
9/9 patients who were not candidates for bypass achieved closure with the combination of Topical Wound Oxygen therapy, weekly applications of HFDS, and conventional wound care consisting of infection control, debridement, off-loading or compression. The average time to closure was 12.7 weeks.

Conclusion
With the use of Topical Wound Oxygen in conjunction with HFDS, we were able to provide an alternative route of care, treatment, and wound closure in a select group of patients who were vascularly compromised, at risk for limb loss, and who were not candidates for bypass surgery. We have found that this technique may provide future benefit for the treatment of challenging, chronic wounds with little potential to heal, based on non invasive studies and no complications.
New Therapeutic Angiogenesis Biomarkers for Chronic Diabetic Foot Ulcers Treated with Transdermal Hyperoxia/Topical Wound Oxygen (TWO$_2$)

Gary F. Scott, Ph.D.
Department of Cell Biology and Genetics,
University of North Texas Health Science Center, Fort Worth, Texas 76107

**Chronic Wound Evaluation**

- Healing is "stalled" in chronic non-healers, typically hyper-inflamed, hyp-oxic.
- Angiogenesis, new capillary synthesis, is required for wound healing to restore blood flow (O$_2$ & nutrients in, waste & toxins out).
- Growth factors, secreted by platelets, neutrophils and macrophages, are required to induce angiogenesis.
  - **Angiogenic biomarkers of new healing are needed:**
    - Endogenous growth factors, ie VEGF, FGF2
    - Functional neo-vascular surface marker, ie Integrin αvβ3
    - Endothelial Progenitor Cell homing signal, ie SDF-1
    - Endothelial secreted vasodilator, ie Nitric Oxide

**Local Molecular & Cellular Abnormalities in a Chronic (non-healing) Diabetic Wound**

- Growth factor and cytokine deficiencies
- Endothelial dysfunction
- Neuropathy: associated with endothelium dependent and independent dysfunction in diabetics predisposed to foot ulceration
- Arterial occlusive disease (PAD): associated with peripheral neuropathy, slower conduction velocity of sensory nerves, depression of autonomic responses
- Abnormalities in fibroblast function
- Abnormalities in extracellular matrix and decreased cellular infiltrate
- Decreased angiogenesis (thus sustained O$_2$ deprivation)

**Oxygen in Tissues and Wounds**

- All nucleated cells use O$_2$ energy metabolism (via mitochondria)
- Epidermis into papillary dermis use transdermal O$_2$
- From blood Hb, O$_2$ diffusion through membranes into is “concentration” dependent
- In wounds, vessels disrupted, so lack O$_2$
- Wound ischemic hypoxia impairs O$_2$-ase enzymes
  - Cytochrome O$_2$-ase for ATP generation, uses 80% of O$_2$ breathed
  - Prolyl hydroxylase for collagen synthesis, req. for angiogenesis
  - Phagocytic O$_2$-ase for bacteria killing via 'respiratory burst'
- Obvious rationale for supplemental O$_2$
- Enforced O$_2$ concentration (TWO$_2$) increases diffusion distance
- Renewed O$_2$ supply can activate repair molecules
  - **Highest priority to restore O$_2$, thus angiogenesis required!!**

**Does Oxygen Restore Healing in Chronic Wounds?**

- What Growth Factors stimulate new blood vessel formation? VEGF & FGF2
- What biomarker do new capillary endothelial cells express that measures functionality? Integrin αvβ3
- What biomarker targets EPCs to injured ischemic tissue? SDF-1
- What O$_2$-sensitive molecules deficient in chronic wounds respond to TWO$_2$? VEGF, FGF2, Integrin αvβ3, SDF-1

**Treatments and Wound Fluid Collection**

- Topical Wound Oxygen Treatments (TWO$_2$) were administered with medical grade oxygen (>95% pure) in a TOCE (Topical Oxygen Chamber for Extremities) for 4 consecutive days, 90 minutes per treatment for 5 weeks.
- Wounds were digitally photographed and wound fluids were collected after treatment on day one and day four of each week’s treatments.
- Fluids from the wound bed were absorbed onto a cotton swab by wiping to collect maximum fluid exudates’ volume. Trimmed swabs containing wound fluids were solubilized in 0.1 M Phosphate Buffered Solution (PBS), fractionated by centrifugation and stored at −20°C for subsequent assay
- Simultaneous quantification of analytes was performed using a customized multiplex enzyme-linked immunoassay (ELISA) at end of 5 weeks of treatment. Total protein in samples was measured.
- Analyte concentration changes per unit of total protein standardized for sample volume variance.
- In current ongoing studies, baseline wound fluid samples are collected weekly for 2 weeks prior to treatment for treatment effect comparison.
Summary of Results of Therapeutic Angiogenic BioMarkers During Transdermal Hyperoxia (TWO₂) Treatments

- Angiogenic Growth Factors
  VEGF & FGF-2 increased significantly
- Integrin αVβ3 (only transiently expressed in new endothelial membrane) increases correspond to angiogenic growth factors' changes
  - confirms formation of new functional capillaries and O₂ re-supply
  - not previously quantified in human wound fluids
- SDF-1 targets BMEPCs (bone marrow-derived endothelial progenitor cells) to injury site (vasculogenesis augments angiogenesis)

Conclusions

- This physiologically relevant set of biomarkers quantify therapeutic angiogenic angiogenesis indicating evidence of renewed activation of dormant cells in chronic wounds, and thus healing.
- These ‘endogenous’ angiogenic biomarkers as surrogate end-points of healing provide evidence allowing comparison of treatment benefits at far earlier timepoints than ultimate clinical endpoints, i.e. full wound closure.
- This mechanism of action analysis of wound responses to transdermal hyperoxia treatment (TWO₂) demonstrates efficacy that reduces costs while improving benefits to a larger number of patients.

References

- Diabetic cellular dysfunctions

- VEGF/FGF2

- Integrin αVβ3

- SDF-1
Topical Wound Oxygen Therapy in the Treatment of Severe Diabetic Foot Ulcers: A Prospective Cohort Study

Blackman E¹, Moore C¹, Frye C²
¹ St. Catharine’s Wound Clinic, Ontario, Canada, ² AOTI Ltd., Galway, Ireland

Introduction

Diabetic foot ulcers (DFU) are frequent, difficult to treat and show high rates of complications.

We examined the clinical efficacy of a unique pressurized topical oxygen therapy (TWO2) device in an outpatient setting in 28 patients with severe diabetic foot ulcers (DFU). Patients visiting a community wound care clinic for treatment of severe DFU’s were offered TWO2 or advanced moist wound treatment (AMWT).

Method

TWO2 patients were treated daily for 60-minutes 5 times a week. The device delivered humidified medical grade oxygen with pressure cycles between 5 and 50 mb.

Results

The primary endpoint was complete ulcer closure after 90 days. 28 patients were included into the study. The TWO2 treatment group recruited more severe wounds. The TWO2 treatment group had significantly more complete ulcer closures after 90 days than the AMWT group (14/17, 82.4%, median 56 days vs. 5/11, 45.5%, median 93 days; (p=0.04)). There was no reoccurrence at the ulcer site after 24 months follow up in either group.

Conclusion

Patients with severe DFU’s treated with TWO2 demonstrated significantly higher and faster healing rates with no ulcer reoccurrence after two years compared to AMWT. TWO2 has the potential to provide substantial quality of life and cost savings benefits to both patients and the health care system as a whole.
The use of Topical Wound Oxygen (TWO2) on Complex Recalcitrant Wounds in Multi-Morbid Patients

Authors: Dr. Bruce Levine¹, Dr. Christian Frye²  ¹ Harbor Foot and Ankle, San Pedro, CA  ² AOTI Ltd, Galway, Ireland

Introduction

Patients suffering from chronic wounds often have multiple chronic conditions that impair wound healing.

Methods and Results

Topical Wound Oxygen (TWO2), manufactured by AOTI Ltd, Ireland works by delivering 100% oxygen at pressure cycles between 5 and 50mbar to enhance the partial oxygen pressure in the wound tissue. We have treated approximately 50 patients with a new therapy working with topical pressurized oxygen, and present data on 14 ulcers from 10 patients: 11 DFU and 3 Venous Stasis which had been unresponsive to conventional wound care and/or NPWT.

Patients received current standard best practice wound care techniques including infection control, debridement, offloading or compression therapy plus the addition of Topical Pressurized Oxygen therapy. 8/10 patients had history of Renal Disease, 4/10 had history of PVD and 8/10 were chronic smokers.

Results

10/10 patients achieved closure on 14 previously non-responsive ulcers. Wound are averaged 8.8 cm² (4.8-13.6) with average time to closure being 11.5 weeks (8-15) and average number of treatments to closure at 33 (19-41).

Conclusion

In these 10 extremely complicated cases, all associated with multiple co-morbidities, the addition of TWO2 proved to be a valuable adjunctive therapy with good results in healing their recalcitrant wounds and more importantly, the maintenance of the patients’ functional status.
First Experience in the Treatment of Chronic Venous Ulcers with Topical Wound Oxygen (TWO\textsubscript{2}) in an Out-Patient setting in Latvia

Aleksandra Kuspeło

**Aim**
We want to share our first experience with TWO\textsubscript{2} in Latvia using a topical oxygen chamber using cycling pressure as an additional method in the treatment of venous ulcers.

**Method**
The patients were treated daily with TWO\textsubscript{2} therapy for 60 five times a week. Prior to each treatment, the patients wound dressings were removed and the wound bed was irrigated with a normal saline solution. The TWO\textsubscript{2} device delivered humidified medical grade oxygen at a cycling pressure between 5 and 50mbar. After each treatment patient received compression stockings of the 2nd functional class or short-stretch compression bandage. Weekly assessments of the wound as well as pictures were taken to document the wound area, volume and changes in each from the previous assessment.

**Results**
We treated 8 patients in total. Four patients with chronic atrophic ulcers of venous aetiology completed treatment with a full ulcer epithelisation (number of treatments - from 13 to 21). Four additional patients with venous ulcers are still receiving treatment as not all patients started at the same time. These patients received 8 to 13 treatments so far and all show good progression of the wound.

**Conclusion**
First experience of using TWO\textsubscript{2} is very positive. Patients with severe venous ulcers benefit from treatment with TWO\textsubscript{2} and show remarkable wound closure rates.
Treatment of a Chronic Stage IV Pressure Ulcer using Topical Wound Oxygen (TWO\textsubscript{2}) Therapy

Anku, Comfort RN, Dr. Christian Frye\textsuperscript{2}  
\textsuperscript{1} Post Inn Village, Toronto, Canada, \textsuperscript{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\textsubscript{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\textsubscript{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\textsubscript{2} treatment, the wound was treated with Silversorb and Betadine then redressed with standard gauze dressing. The TWO\textsubscript{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 cm\textsuperscript{2} with a volume of 109.2 cm\textsuperscript{3}. 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 cm\textsuperscript{2} and volume to 11.38 cm\textsuperscript{3}. TWO\textsubscript{2} therapy was discontinued during the hospitalization. TWO\textsubscript{2} resumed one month later; with an area of 5.28 cm\textsuperscript{2} and volume of 12.5 cm\textsuperscript{3}. After 2 additional weeks of therapy, the wound had 100\% closure.

Observations:

1. TWO\textsubscript{2} improves local tissue perfusion  
2. TWO\textsubscript{2} softens necrotic tissue and enhances debridement  
3. TWO\textsubscript{2} eliminates maceration  
4. TWO\textsubscript{2} reduces nursing intervention time

Conclusion

Patients with severe chronic wounds benefit from the treatment with TWO\textsubscript{2} and show remarkable wound closure rates.
Case Study

This is a case study of a 59-year old patient who was admitted to our hospital due to progressively deteriorating condition and no appetite. The patient had a hemiparesis on his left side due to a meningitis as a child as well as a general exanthema due to an allergic reaction on antibiotic treatment. Laboratory analyses revealed significant signs of infection. The patient developed a forefoot phlegmone that started from a venous ulcer at his right inner leg that had been there since years. Rapidly the patient developed a sepsis that made intermittent ventilation as well as dialyses and high dose catecholamines necessary. The ventral muscle compartments of the forefoot were incised followed by an open wound therapy for 4 weeks. As laboratory infections signs started to increase again, the wound was revised followed by 4 weeks of Negative Pressure Treatment (NPT). The lower leg had a significant edema at this point. The wound has granulated well but showed a great deal of sludge. Wound healing had stalled with no further signs of epitheliasation. Therefore we started TWO2 therapy at a duration of 3-6 hours per day 8 Week after the first surgery. Even though massive substitution of liquids was still necessary the edema of the lower leg and the foot was reduced remarkably. The wound epithelialised quickly. The venous ulcer at the lower leg that was responsible for the sepsis healed within 30 days during intensive care. The incision on the foot showed good granulation. In total the patient spent 14 weeks in intensive care! The patient was dismissed from intensive care in a center of neurologic rehabilitation.

Conclusion

In an intensive care setting the administration of TWO2 is well tolerated. It promotes excellent healing in complex wounds and seem to be a valuable adjunctive therapy.
Limb Salvage with Topical Wound Oxygen (TWO₂) – Two Cases of Complex Wounds in Multimorbid Patients and Imminent Major Amputation

Dr. Helmut Adler¹, Dr. Christian Frye²
¹ Klinikum Forchheim, Forchheim, Germany, ² AOTI Ltd, Galway, Ireland

Introduction

Patients suffering from chronic ischemic wounds often have multiple chronic conditions that impair wound healing. We present two cases we treated with a new therapy working with oxygen and cyclical pressure.

Methods and Results

Topical Wound Oxygen (TWO₂) from the manufacturer AOTI Ltd, Ireland works with purified oxygen and pressure cycles between 5 and 50mbar to enhance the partial oxygen pressure in the wound tissue.

Case 1:

A 64 year old male patient had an autologous femoro-popliteal bypass surgery done 4 weeks prior to admission. We saw the patient with a complete necrosis of the skin on dorsal site of the foot. Surgical removal of necrosis and resection of compartment on back of foot as well as amputation of toes were performed. We continued therapy with negative pressure therapy (NPT) and intermittent TWO₂ therapy. After skin grafting NPT and intermittent TWO₂ therapy for 7 days was done. After stopping NPT, TWO₂ treatment alone was for 10 days before dismissal home.

Case 2:

72 year old male diabetic patient with AVK developed a gangrenous forefoot. Prior to admission to surgical ward therapy with prostavasin was done. There were no possibilities to improve arterial vascular status via surgical procedures. A transmetatarsal amputation with repeated debridement was performed followed by negative pressure therapy and resection of necrotic tissue. After 6 weeks of no further improvements we started with TWO₂ for 6 days. The wound granulated well and we decided to skin graft followed by negative pressure and TWO₂ therapy on days of dressing changes for 6 days. After 13 days of solely TWO₂ the wound granulated well and the patient was dismissed to rehabilitation.

Conclusion

In these two complicated cases both associated with severe comorbidities TWO₂ proved to be an valuable adjunctive therapy with good results in healing and more important to maintain the functional status by avoiding major amputation.

Presented at EWMA 2010 May 26-28, Geneva Switzerland
This is a case of a 77 yr/o Male with a Hx of CHF, Afib, COPD, HTN, GERD who presented with recurrent Stasis Ulcerations for the last 40 years. For the last 15 months, the patient has not been able to heal the ulcers and can not wear compression hose due to discomfort and drainage.

**Left Leg:**
Venous Stasis Ulcerations x 2
Grade IIA: 11.2 x 5.5 cms / 8 x 4 cms
Radiographs: unremarkable
WBC 6.2, Sed Rate 20, CRPH .8
ABI: Right .70 Left .68
Venous Duplex Scan: Negative
C&S: S. aureus (negative MRSA)
Keflex 250 QID x 14 days

**Treatment and Methodology:** The 2 large stasis ulcerations c/o non-viable, dry wound bases. Topical Wound Oxygen was initiated first for 90 minute sessions bid followed by Santyl dressings. Wound Conversion took place approximately 5 weeks afterwards where the wound base became granular and Grafix Prime was applied. Of special note was the immediate decrease in pain and restoration of normal skin coloration about the ulcerations.

**CONCLUSION:** The Grafix Prime Mesenchymal Stem Cells were left in place during the TWO2 treatments and changed weekly. The wound fully healed at 13 weeks and the patient was then sized for custom hose. Both Modalities worked extremely well in this case study and should be highly considered in the treatment of chronic venous stasis leg wounds.
The Use of Topical Wound Oxygen (TWO2) for the Treatment of an Ischemic, Dehisced Open Partial 4th Ray Amputation with elevated CRPH Levels and Kidney Disease

This is a case of a 77 yr/o male with a hx of DM, CHF, CKD, HTN, PAD and PVD who underwent a Partial 4th Ray Amputation secondary to Osteomyelitis and wet Gangrene. A collaborative effort was established with Infectious Disease for chronic infection.

Francis Derk, DPM, CDR USN
STVHC: Chief Podiatry Services
UTHSC: Adjunct Clinical Faculty

ABIs: Right and Left: Non-compressible (N/C)
TBIs: Right and Left: Non-compressible (N/C)
Admission: UT Grade IVD, WBC 17, Sed Rate 100
C-Reactive Protein (CRPH) 9,787, DVT prophylaxis

CULTURES: P. Aeruginosa, C Braakii mod growth
Proximal Margin: Permanent Specimen Clean

TWO2 Started 16 days following DIC

Treatment: S/P day 4 D/C, WBC 6.4, CRPH 4.2
Antibiotics: Cipro 500 mgs qd / Amoxacillin 250 bid
(Renally dosed for 1 week: Stage III Kidney Disease)

S/P 3 weeks Sinus Tract/Open Wound

CRPH 2.7

Topical Wound Oxygen initiated week 3
CRPH 3.5

S/P 5 weeks
CRPH 2.7

S/P 5 weeks
CRPH 1.3

S/P 6 weeks
CRPH .9

S/P 7 weeks
CRPH .7

S/P 9 weeks

S/P 11 weeks

RESULTS: The CRPH level dropped to WNL at approximately week 6 and wound conversion was attained to a UT Grade II A with full healing at week 9. Follow up X-rays and labs were unremarkable and the patients kidney function was still operable.

METHODOLOGY: The Open Wound still probed deeply to bone (UT Grade IV D) s/p 3 weeks with ongoing concerns of an elevated CRPH of 3.5 and ischemia. Infectious Disease extended both oral antibiotics to 4 weeks and monitored the patient closely.

CONCLUSION: With the assistance of Infectious Disease as well as TWO2, the author was able to successfully heal the wound despite multiple comorbidities including CKD and a poor vascular spectrum. This case study demonstrates the effectiveness of not only TWO2, but also comanagement by a multi-service approach.
The Use of Topical Wound Oxygen (TWO2) in a Severe Lower Extremity Staph Infection Status Post Incision and Drainage

This is a case of a 48 yr/o male with a hx of Afib, GERD, and HTN who presented to the Emergency Room with a CC of an ongoing infection of the Right Lower Extremity following a prior admission for Cellulitis. Last admission Methicillin Sensitive S. Aureus (MSSA) infection of the Right leg 7 days S/P.

Francis Derk, DPM, CAPT, USN
STVHCS: Chief Podiatry Services
UTHSC: Adjunct Clinical Staff

*PRIOR ADMISSION: MSSA
IV Cefazolin 2 gms D5W 50/50
Oral Keflex: 500 mgs qid x 14

**NEW ADMISSION: MSSA and Strep Pyogenes
IV Cefazolin 2 gms D5W 50/50
Mini-bag infusion 30 min q 8 hrs
Oral: Keflex: 500 mgs qid x 14

CLINICAL PRESENTATION: multiple pustules of the lower Right Lower Extremity, Cellulitis, purulent drainage
X-rays: unremarkable and no gas involvement
CT Scan: superficial soft tissue fluid collections diffuse
WBC 15.1, CRPH 9, Sed Rate 24, Neutrophils 82.3, T 103
*Fluctuance dorsal foot, proximal 1/3 leg medially
*Lymphadenopathy: groin, lower extremity edema
Blood Cultures: negative

CONCLUSION: The author presents a case of a Fulminant Right Lower Extremity Infection following a recent 1 week hospitalization and discharge for Cellulitis and blisters of the Right Lower Extremity. TWO2 was very beneficial s/p I&D and should be considered for complicated, open post operative wounds.

TREATMENT: An emergent, full thickness Incision and Drainage was performed from the proximal 1/3 of the leg to the distal Right foot. Approximately 80 ccs of purulent drainage was evacuated and deep cultures were taken (noted above results). The wound was partially closed and following post op day 3, Topical Wound Oxygen was initiated for 90 minute sessions BID. Iodosorb dressings were utilized.

The Patient was discharged after 3 days (WBC 5.5, CRPH 7) and switched to Keflex 500 mgs QID x 14 days non weight bearing. No further complications were encountered and full recovery noted at 7 weeks.

CONCLUSION: The author presents a case of a Fulminant Right Lower Extremity Infection following a recent 1 week hospitalization and discharge for Cellulitis and blisters of the Right Lower Extremity. TWO2 was very beneficial s/p I&D and should be considered for complicated, open post operative wounds.
A Randomized Controlled Trial to Evaluate Different Treatment Regimes with Topical Wound Oxygen (TWO₂) on Chronic Wounds

Aburto I¹, Frye C²
¹ Instituto Nacional de Heridas (INH), Santiago, Chile, ² AOTI Ltd., Galway, Ireland

Introduction
Chronic wounds on the lower leg and foot are frequent, difficult to treat and show high rates of complications (1). After very positive results with a unique pressurized topical oxygen therapy (TWO₂) device in other studies (2,3) we investigated whether 4 weeks of TWO₂ treatment and consecutive 8 weeks of advanced moist wound treatment (AMWT) is equally effective in healing chronic wounds as continuous treatment with TWO₂.

Method
The randomized, controlled study was conducted at the National Wound Institute in Santiago de Chile. In an outpatient setting with patients with severe diabetic foot ulcers (DFU) (n=20) and chronic venous ulcers (CVU) (n=20) all patients received TWO₂ for a period of one month. Then the groups were randomized to continue with TWO₂ (TWO₂-TWO₂ group) or receive AMWT for 2 more month (TWO₂-AMWT group). TWO₂ patients were treated daily for 2 hours 5 times a week. The device delivered humidified medical grade oxygen with pressure cycles between 5 and 50 mbar. Dressing changes in the control group were performed according to best practice at a minimum of twice a week. The primary endpoint was complete ulcer closure after 90 days.

Results
The majority (82%) of the patients were referred to the study center for minor or major amputation. All of these patients improved under the therapy and no patient underwent amputation. Patients were comparable concerning age, size of the wound and duration of the wound. 90% of the DFU patients in the TWO₂-TWO₂ group healed within 90 days vs. 40% in the TWO₂-AMWT group. Patients with CVU had 50% healing vs. 30%, respectively.

Conclusion
Patients with complicated ulcers benefit from the treatment of topical localized oxygen (TWO₂). Continuous TWO₂ treatments for 12 weeks showed significant better outcomes than a shorter TWO₂ treatment regime of 4 weeks followed by AMWT.

A Randomized Controlled Trial to Evaluate Different Treatment Regimes with Topical Wound Oxygen (TWO$_2$) on Chronic Wounds

(Oral Abstract No. 181)

Isabel Aburto T., Directora
Instituto Nacional de Heridas, Chile

Noordwijkherout – May 13, 2011
Oxygen is Needed to Heal

- Energy metabolism $^{1,2}$
- Neovascularisation $^{1,3,4,5}$
- Cell migration $^2$
- Syntheses of collagen $^{6,7}$
- Enhancement of fibroblasts $^8$
- Inhibition of bacterial growth (infection) $^{10,11}$

3. Fries, Mutat Res. 2005  
# Chronic wounds lack oxygen

## The oxygen dilemma of chronic wounds

<table>
<thead>
<tr>
<th>Chronic wounds lack O2</th>
<th>Chronic wounds have a higher demand of O2</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Disrupted or compromised vasculature</td>
<td>• O2 is needed to heal</td>
</tr>
<tr>
<td>– Diabetes</td>
<td>- Syntheses of collagen</td>
</tr>
<tr>
<td>– Arterioscleroses</td>
<td>- Building of Extra Cellular Matrix (ECM)</td>
</tr>
<tr>
<td>– Trauma</td>
<td>- Neovascularization</td>
</tr>
<tr>
<td>• Edema</td>
<td>• And to fight infection</td>
</tr>
</tbody>
</table>

---

1Sen CK, Wound Repair and Regeneration 2008
Cycling topical wound oxygen device (TWO₂)

1. Raises Wound Tissue Oxygen Levels
2. “No-touch” compression
3. Lymph drainage due to cycling pressure
## Peer Reviewed Controlled and Randomized Studies on Topical Oxygen

<table>
<thead>
<tr>
<th>Author</th>
<th>Published</th>
<th>Indication</th>
<th>Randomized</th>
<th>Patients (Treatment/Control)</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aburto</td>
<td></td>
<td>20 Diabetic, 20 Venous</td>
<td>Yes</td>
<td>20/20</td>
<td>Diabetic: 90 vs. 40% Venous: 50 vs. 30% heeled in 12 weeks</td>
</tr>
<tr>
<td>Blackman</td>
<td>2010</td>
<td>Diabetic</td>
<td>No but controlled</td>
<td>17/11</td>
<td>82% vs. 45% healed (12 wks)</td>
</tr>
<tr>
<td>Nie</td>
<td>2010</td>
<td>Burns</td>
<td>Yes</td>
<td>23/18 (Total 85)</td>
<td>Burns: Wound healing rates 85% vs. 68%</td>
</tr>
<tr>
<td>Sultan</td>
<td>2010</td>
<td>Venous</td>
<td>No but controlled</td>
<td>46/37</td>
<td>3 year follow up. 80% vs. 14% remained heeled in 36 month</td>
</tr>
<tr>
<td>Tawfick</td>
<td>2009</td>
<td>Venous</td>
<td>No but controlled</td>
<td>46/37</td>
<td>80% vs. 35% healed (12 wks)</td>
</tr>
<tr>
<td>Heng</td>
<td>2000</td>
<td>Mostly diabetic</td>
<td>Yes</td>
<td>13/27</td>
<td>90% vs. 22% healing (16 weeks)</td>
</tr>
<tr>
<td>Leslie</td>
<td>1988</td>
<td>Diabetic</td>
<td>Yes</td>
<td>12/16</td>
<td>&gt;50% wound reduction in both groups but no difference but after only 2 weeks</td>
</tr>
</tbody>
</table>
Chile

- 4,275 km long, on average 180km wide
- Santiago has 5.5m inhabitants (50% of total population)
- Corruption Index better than most European countries
- Highest per capita income and highest export in South America
Setting

- The study was planned and organised at the National Wound Institute in Chile
- In conjunction with the University Major and the Health Ministry
- Investigator driven trial
Study design: Prospective Randomized Trial

Diabetic ulcers: N=20
- Randomization
- 4 weeks topical oxygen
- Standard wound care for 8 weeks N=10

Venous ulcers: N=20
- Randomization
- 4 weeks topical oxygen
- Standard wound care for 8 weeks N=10

Hypertensive ulcers: N=5
- Randomization
- 4 weeks topical oxygen
- Standard wound care for 8 weeks N=3

- Topical oxygen for 8 weeks N=2

Ulcera hipertensiva:
- existence of hypertension, absence of arterial obstruction (palpable peripheral pulses), absence of venous insufficiency,
- presence of a superficial ulcer in the anterior area of the leg
Methods

Inclusion criteria
- Healing less than 1cm² in last 4 weeks
- Patients were referred from the metropolitan area of Santiago
- No occlusive arterial disease (ABI < 0.5)
- Ulcers > 5 cm and < 10 cm in diameter

Exclusion criteria
3 or more of the following conditions
- Obesity (BMI > 35)
- Vasculitis
- Radiotherapy
- Chemotherapy
- Chronic renal insufficiency (creatinine > 2.0 mg%)
- Hepatic impairment (Child-Pugh-Score B or C)
- Systemic autoimmune diseases
- Corticosteroids for a period longer than a month.
- Hypertension (> 140/90)

All patients were assessed by a vascular surgeon at baseline
Assessments

• Biweekly assessment of wound size
• Vascular assessment at baseline and every 4 weeks by vascular surgeon
• Geriatric assessment at baseline and end of study
• Social assessment by social worker to assess socioeconomic status
• Psychological assessment by psychologist to evaluate depression
  – Canberra Interview for the Elderly (standardized diagnostic interview for dementia and depression), Christensen et al, 1995
• Lab: Hb, albumin, glucose, creatinine, uric acid, hematokrit, VHS, prothrombine, WBC count
• Quality of life:
  – Dermatology Life Quality Index: DLQI(AY Finlay, GK Khan, 1992)
  – Barthel Index, Mahoney/Barthel, 1965
  – IADL: instrumental activities of daily living, (Lawton and Brody, 1969)
Study treatments

Topical oxygen
- Daily 2 hours of treatment (Monday-friday)
- Depending on exudate gauze dressing, hydrogel, nonadhesive transparent dressing, alginate or foam.
- Healthy lifestyle education at each visit

Control group
- Treatment according to protocol of National wound Institute
- Depending on exudate gauze dressing, hydrogel, nonadhesive transparent dressing, alginate or foam.
- Healthy lifestyle education at each visit
None of the 37 patients underwent amputation during the study.

37/45 (82%) of patients were suggested for amputation.

• Baseline – Referred for Amputation
Results - Depression

- 100% of patients had depression at start of study
- 35 patients (78%) had required psychiatric or psychological care.
- 22 patients (49%) had lost contact with family members
- 39 patients (87%) felt discriminated in public places,
  - of whom 18 patients (46%) attributed it to bad smell.
- 41 patients (91%) had decreased social activities because of bad smell and ulcer pain
Results - Infection

- On admission to the study, 30 of 45 patients (66%) had clinical infection.
- After 4 weeks of topical oxygen 4% had signs of infection.
Results - Pain

Diabetic ulcers  
\( n=20 \)

Venous ulcers  
\( n=20 \)

Hypertensive ulcers  
\( n=5 \)

Base 1st month 2 month 3 month

12 wks TWO
4 wks TWO 8 wks SWC

12 wks TWO
4 wks TWO 8 wks SWC

12 wks TWO
4 wks TWO 8 wks SWC
Pain medication

- 93% of patients used analgesics at baseline, and 16% after 3 months
Healing
Kaplan-Meier Survival analyses

Diabetic ulcers
n=20

Venous ulcers
n=20

Hypertensive ulcers
n=5

Diabetes 12 wks TWO
Diabetes 4 wks TWO 8 wks SWC

Venous 12 wks TWO
Venous 4 wks TWO 8 wks SWC

12 wks TWO
4 wks TWO 8 wks SWC
## Strengths and Limitations of the Study

<table>
<thead>
<tr>
<th>Strengths</th>
<th>Limitations</th>
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</thead>
<tbody>
<tr>
<td>Randomized controlled study</td>
<td>“Dose-Response” Design</td>
</tr>
<tr>
<td>High quality data</td>
<td>Small number of patients</td>
</tr>
<tr>
<td>Multidisciplinary team</td>
<td></td>
</tr>
</tbody>
</table>
Conclusions

• Continuous TWO2 treatments for 12 weeks showed better outcomes than a shorter TWO2 treatment regime of 4 weeks followed by standard wound care

• TWO2 had significant effects on wound associated conditions:
  – Pain decrease
  – depression decreased significantly, most patients stopped using sedatives and analgesics
  – clinical infections

• In conclusion we believe that topical oxygen is a valuable contribution in the treatment of hard to heal chronic ulcers
The Team

Isabel Aburto – Director – Instituto Nacional de Heridas
Dr. Rodrigo Julio - Cirujano Vascular - Instituto Nacional de Heridas
Patricia Morgado - Enf. Programa Cardiovascular – Minsal
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PERSPECTIVE ARTICLE

Wound healing essentials: Let there be oxygen

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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 O2 in the perioperative period reduces the incidence of postoperative infections. Correction of wound pO2 may, by itself, trigger some healing responses. Importantly, approaches to correct wound pO2 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 O2 to wound healing occurs at many levels: diagnostic, preventive, and therapeutic. From a diagnostic standpoint, measurements of wound oxygenation (transcutaneous O2 measurements or TCOM) are commonly used to guide treatment planning such as amputation decision. In preventive applications, optimizing wound perfusion and providing supplemental O2 in the perioperative period reduces the incidence of postoperative infections. Correction of wound pO2 may, by itself, trigger some healing responses. More importantly, approaches to correct wound pO2 favorably influence outcomes of other therapies such as responsiveness to growth factors and acceptance of grafts. This leads to the concept of correction of wound hypoxia as adjunct to other therapeutic modalities. 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 O2 sensing and response with the goal to illuminate salient complexities and perform critical analysis of what should help improve clinical outcomes in response to O2-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 O2-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 (pO2) compared with the pO2 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. 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, 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.

Three major factors may contribute to wound tissue hypoxia: (i) peripheral vascular diseases (PVDs) garrotting O2 supply, (ii) increased O2 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
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. In this context, it is also important to appreciate that point measurements 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 homogeneous 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. 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 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. 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. 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\textsubscript{2} supply to the wound tissue. Compromised pulmonary health, loss of hepatic function, hemodilysis, anemia, altitude hypoxemia, nitroglycerin therapy, nasal packing, critical illness, pain, and hypothermia are some examples of conditions associated with arterial hypoxemia. Vasoconstricting drugs may contribute to tissue hypoxia as well.57

**High demand: increased demand of the healing tissue**

Mitochondrial respiration is responsible for more than 90% of O\textsubscript{2} consumption in humans. Cells utilize O\textsubscript{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.58 Increased energy demand of the healing tissue leads to a hypermetabolic state wherein additional energy is generated from oxidative metabolism increasing the O\textsubscript{2} demand of the healing tissue.59 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.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\textsubscript{2} and its derivatives in wound healing as discussed below.

Absolute requirements for O\textsubscript{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\textsubscript{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\textsubscript{2} across the entire physiologic range, from 0 to hundreds of mmHg. The K\textsubscript{m} for O\textsubscript{2} for this reaction is approximately 25 and the V\textsubscript{max} is approximately 250 mmHg, suggesting that new vessels cannot even approach their greatest possible rate of growth unless the wound tissue pO\textsubscript{2} is high.66 Angiogenesis is directly proportional to pO\textsubscript{2} in injured tissues.67 Hypoxic wounds deposit collagen poorly and become infected easily, both of which are problems of considerable clinical significance.68,69

**High demand: increased production of reactive species**

**Phagocytic NADPH oxidases**

Sbarra and Karnovsky’s 1959 discovery of the leukocyte oxidase68 in phagocytes came into limelight in the late 1970s, when the pioneering works of Bernard Babior linked the explosive production of superoxide ions (O\textsubscript{2}–) by leukocyte oxidase to bacterial killing.70 During phagocytosis of microbial intruders, professional phagocytes of our innate immune system increase their O\textsubscript{2} consumption through the inducible activity of NADPH oxidase (NOX) that generates O\textsubscript{2}– and H\textsubscript{2}O\textsubscript{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\textsubscript{2} → NADP++2O\textsubscript{2}–+H\textsuperscript{+}). The O\textsubscript{2}– then rapidly dismutates to H\textsubscript{2}O\textsubscript{2}. Approximately 98% of the O\textsubscript{2} consumed by wound neutrophils is utilized for respiratory burst.72 NADPH oxidase supports macrophage survival73 and enables dead cell cleansing by phagocytosis.74 Appropriate infection management may therefore spare precious O\textsubscript{2} at the wound site, which would otherwise be utilized via respiratory burst.75 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\textsubscript{2}•– in the lumen. It is generally accepted that this
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The field of redox signaling was thus born\textsuperscript{102–105–107} with a dedicated international peer-reviewed journal (http://www.liebertpub.com/ars). Today, the concept that reactive derivatives of \( \text{O}_2^* \) may serve as signaling messengers has revolutionized cell biology\textsuperscript{108–123} and has led to the concept of redox-based clinical therapeutics\textsuperscript{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\textsuperscript{127} Mox1 or p65Mox was described as encoding a homolog of the catalytic subunit of the \( \text{O}_2^* \)-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\textsuperscript{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\textsuperscript{131–135}. NOX enzymes also contribute to a wide range of pathological processes. NOX deficiency may lead to immunosuppression, lack of otoconogenesis, or hypothyroidism. Increased NOX activity also contributes to a large number of pathologies, in particular cardiovascular diseases and neurodegeneration\textsuperscript{136}. Thus, optimal generation of \( \text{O}_2^* \) 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\textsuperscript{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 \( \text{H}_2\text{O}_2 \) production\textsuperscript{56}. This response is also conserved in plants\textsuperscript{57}. Wound fluid from healing tissues contains the highest concentration of \( \text{H}_2\text{O}_2 \) compared with all other bodily fluids\textsuperscript{56,138}. Of note, selective decomposition of \( \text{H}_2\text{O}_2 \) at the wound site using catalase overexpression approaches impairs the healing process demonstrating the key significance of \( \text{H}_2\text{O}_2 \) in wound healing\textsuperscript{56}.

Importantly, catalase-dependent decomposition of \( \text{H}_2\text{O}_2 \) generates \( \text{O}_2 \) as an end-product. Thus, molecular \( \text{O}_2 \) is not sufficient if NADPH oxidase-dependent \( \text{O}_2 \) consumption and redox signaling is impaired. How redox signals may contribute to

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\textsuperscript{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\textsuperscript{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 \( \text{H}_2\text{O}_2 \) may serve as signaling messengers\textsuperscript{102–104}.
tissue repair has been recently reviewed elsewhere\textsuperscript{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\textsubscript{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-\(\beta\)) and numerous molecular mechanisms (e.g., leukocyte recruitment, cell motility, integrin function), which rely on redox signaling.\textsuperscript{57,139,140}

Collagen deposition provides the matrix for angiogenesis and tissue remodeling. Maturation of collagen is O\textsubscript{2} dependent. Of the O\textsubscript{2}-dependent enzymatic processes, the rate of collagen synthesis is reflected by the rate at which prolyl hydroxylation occurs.\textsuperscript{141} Collagen synthesis is half-maximal (\(K_{\text{m}}\) using Michaelis–Menton equation) at a \(pO_2\) of 20–25 mmHg.\textsuperscript{58,142} with \(V_{\text{max}}\) at levels approaching 250 mmHg. This represents levels of O\textsubscript{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.\textsuperscript{143–145}

**Nitric oxide (NO) synthases**

NO is widely recognized as a major signaling messenger that drive numerous aspects of (patho)physiology.\textsuperscript{146–149} O\textsubscript{2} consuming NO synthases (NOS) catalyze NO formation from the amino acid \(L\)-arginine. The reaction of NOS with O\textsubscript{2} is fast and takes place within several steps.\textsuperscript{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 \(O_2^-\) and \(H_2O_2\).\textsuperscript{151} The key significance of NO in wound healing has been reviewed elsewhere.\textsuperscript{152,153}

In the context of this article, it is important to note that O\textsubscript{2} is often the overlooked substrate in NO synthesis. To date, there has been little consideration of the role of O\textsubscript{2} tension in the regulation of NO production associated with wound healing. Tissue O\textsubscript{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.\textsuperscript{154} The preliminary observation that hyperbaric oxygen (HBO) therapy may significantly increase NO levels is therefore understandable.\textsuperscript{155}

Once generated, the biological significance of NO also depends on the tissue oxygenation status.\textsuperscript{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\textsubscript{2}. Concentrations of NO too low to inhibit respiration can trigger cellular defense response mechanisms. Inhibition of mitochondrial respiration by NO at low O\textsubscript{2} concentrations can cause so-called “metabolic hypoxia” and divert O\textsubscript{2} toward other oxygen-dependent systems. Metabolic hypoxia refers to a state wherein although O\textsubscript{2} is available the cell is unable to utilize it for respiration.\textsuperscript{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-1\(\alpha\), thus leading to a situation in which the cell may fail to register hypoxia. Furthermore, in a wound setting where O\textsubscript{2}\(^{2-}\) production is highly active, NO is likely to generate peroxynitrite that can affect the action of key enzymes, such as mitochondrial complex I, by \(\beta\)-nitrosation.\textsuperscript{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.\textsuperscript{158} Under conditions of normoxia, NO may attenuate the ubiquitination of HIF-1\(\alpha\) and thus abrogate binding of von Hippel-Lindau (pVHL) to HIF-1\(\alpha\).\textsuperscript{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-1\(\alpha\) synthesis by NO.\textsuperscript{160} Although speculative, different redox-active products, derived from chemically distinct NO donors, use divergent transmission systems to stabilize/express HIF-1\(\alpha\).\textsuperscript{160} Under conditions of hypoxia, NO and its derivatives inhibit hypoxia-induced HIF-1\(\alpha\) accumulation.\textsuperscript{158} In light of the observation that NO attenuates PHD activity under normoxia to stabilize HIF-1\(\alpha\), 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-1\(\alpha\), which accounts for protein stability, is needed for NO and its derivatives to reverse hypoxic HIF-1\(\alpha\) stabilization.\textsuperscript{161} Several mechanistic hypotheses have been proposed to explain how NO impairs accumulation of HIF-1\(\alpha\) under hypoxia.\textsuperscript{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.\textsuperscript{159,162,163} 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 \(O_2^-\) and NO represents a primary biochemical path in vivo.\textsuperscript{162} Flux rates of NO and \(O_2^-\) as well as the presence of antioxidant enzymes, can modulate HIF-1\(\alpha\) stabilization.\textsuperscript{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\textsuperscript{157} becomes critically important in the context of planning NO-based therapeutics.

**THE NORMOXIC SETPOINT AND OXYGEN SENSING**

Cellular O\textsubscript{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: Drosofila Period (Per), the human aryl hydrocarbon receptor nuclear translocator (Arnt), and Drosophila Single-minded (Sim). 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-1\(a\) (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-1\(\beta\) and an oxygen-regulated subunit HIF-1\(\alpha\) (or its paralogs HIF-2\(\alpha\) and HIF-3\(\alpha\)). The transcriptional role of HIF is primarily dependent on the stabilization of HIF-1\(\alpha\) or its paralogs under hypoxic conditions. Under \(O_2\)-replete conditions HIF-1\(\alpha\) is very labile.\(^{176}\) Molecular \(O_2\) targets HIF for degradation by posttranslational hydroxylation at specific prolyl residues within the \(\alpha\) subunits. Hydroxylation at two prolyl residues within the central degradation domain of HIF-1\(\alpha\) increases the affinity for the pVHL E3 ligase complex by at least three orders of magnitude, thus directing HIF-\(\alpha\) 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-\(\alpha\) 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-1\(\alpha\) fails to form a fully active transcriptional complex. Thus, HIF prolyl hydroxylase 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

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

<table>
<thead>
<tr>
<th>Erythropoiesis/iron metabolism</th>
<th>Cell survival/proliferation</th>
<th>Angiogenesis</th>
<th>Vascular tone</th>
<th>Glucose metabolism</th>
<th>Matrix metabolism</th>
</tr>
</thead>
<tbody>
<tr>
<td>EPO</td>
<td>IGF-2</td>
<td>VEGF</td>
<td>NOS2</td>
<td>HK1,2</td>
<td>MMPs</td>
</tr>
<tr>
<td>Tf</td>
<td>TGF-α</td>
<td>Leptin</td>
<td>HO1</td>
<td>LDHA</td>
<td>PAR/PAI</td>
</tr>
<tr>
<td>Tfr</td>
<td>ADM</td>
<td>TGF-β3</td>
<td>ET1</td>
<td>PKM</td>
<td>Coll PHD</td>
</tr>
<tr>
<td>Ceruloplasmin</td>
<td>BNip3</td>
<td>EG-VEGF</td>
<td>ADM</td>
<td>PFKL</td>
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<tr>
<td></td>
<td>NIX</td>
<td></td>
<td>α$_{1b}$</td>
<td></td>
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<tr>
<td></td>
<td>NDRG2</td>
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</tbody>
</table>

α$_{1b}$, adrenergic receptor; ADM, adenomedulin; AK, adenylate kinase; ALD, aldolase; BNip3, Bcl-2 adenovirus E1B 19kD-interacting protein 3; CA, carbonic anhydrase; Coll PHD, collagen prolylhydroxylases; EG-VEGF, endocrine gland-derived VEGF; ENO, enolase; EPO, erythropoietin; ET, endothelin; GAPDH, glyceraldehyde 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.

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Oxygen and wound healing

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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. 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. 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. 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. 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 underling vascular disorders.

**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” and Carl Scheele’s (1771) “fire air” were soon characterized by Antoine Lavoisier as pure respirable air. Within decades of the first realization that oxygen is the element of life, Brize-Fradin 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. 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 captured the interests of biomedical scientists when in 1969 McCord and Fridovich demonstrated that a metalloenzyme produced H$_2$O$_2$ by combining O$_2^*$ with hydrogen. Today, H$_2$O$_2$ is widely known to function as a cellular messenger. Hypoxia-inducible molecular biomarkers have been characterized enabling us to detect hypoxic insult long before overt signs of oxygen toxicity and adverse clinical symptoms are manifested.

Although marginal hyperoxic challenge may induce favorable responses, a state of tissue oxygenation that far exceeds the normoxic setpoint of a given tissue is a clear risk factor that deserves appropriate attention. 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 hypoxia-induced growth arrest or simply overt oxygen toxicity. One needs to be cautious about too much of a good thing. 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. Moderate hyperoxia increases the appearance of new blood vessels in wounds. In addition to inducing VEGF gene expression, moderate hyperoxia enhances the expression of VEGF proteins and facilitates the release of VEGF from cell-associated stores. Among the factors that may oppose wound healing, extreme hyperoxia causes growth arrest and cell death by a mitochondria-dependent apoptosis pathway. In addition, extreme hyperoxia does pose the threat of oxidative stress.

**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. 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
HIF but does not guarantee transcriptional function. Coexisting extremely hypoxic wounds. Furthermore, it is important 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. 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 prolylase, 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 prolylase may play a role in angiogenesis. Recently it has been demonstrated that proldase inhibits PHD activity to induce HRE-dependent transactivation and facilitate angiogenic signaling. 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 O2 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. 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. Although oxygen toxicity may not be imminently overt, an overdose of O2 is likely to trigger molecular responses such as cell cycle arrest and epigenetic modifications, 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 O2 tension much beyond the period of treatment.

The most fundamental factors in wound care are fluid management, temperature management, pain control, increased arterial O2 tension, the use of appropriate sterile techniques, and administration of prophylactic antibiotics. In addition, numerous cellular and molecular players are required to act in concert to successfully execute wound healing. While examining the efficacy of O2 therapy in wound healing, it is critically important to recognize that O2 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.

**HBO**

HBO therapy represents an effective approach to bolster tissue O2 levels and has been found to benefit wound healing under specific conditions. Importantly, HBO may potentially work synergistically with growth factors such as PDGF to improve the outcomes of ischemic wounds. Because PDGF requires O2-derived H2O2 for successful function, this finding is not surprising. HBO causes sharp elevation in tissue pO2. The administration of two atmospheres of 100% O2 for 2 hours may raise tissue pO2 by 10–20-folds 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 hypoxia through induction of bone marrow NO with resulting enhancement in ischemic limb perfusion and wound healing. HBO may also increase NO levels in perivascular tissues via stimulation of NOS. Exposures to 2.0 and 2.8 ATA O2 stimulate 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 O₂ appeared to be due to an altered cellular redox state. Exposure to 2.8 ATA O₂, but not 2.0 ATA O₂, 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 pO₂ following HBO results in molecular responses such as growth arrest,252–254 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 treatment269–272 and call for HBO dosing regimens where physicians would prescribe the target wound pO₂. This approach would be consistent with the emerging concept of personalized healthcare273 and would require the design of new HBO devices fitted with the capability of real-time mapping of wound O₂ 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 O₂ gas is able to modestly increase the pO₂ of the superficial wound tissue.278 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 O₂ gas in both clinical1,18 as well as preclinical277 settings warrant serious consideration of this approach. Recently, perfluorocarbon droplets encapsulated in aqueous continuous phase has been used as topical O₂ 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 pO₂ was not examined, however.278 Epithelial wound healing is improved by transfer- nal sustained-delivery treatment with 100% O₂.14 A recent clinical study testing the effects of topical O₂ 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 O₂ is known to induce VEGF; 15,63,218 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.279 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 pO₂,277 and cannot match the large increases in tissue pO₂ typically noted in response to HBO.252–254 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 pO₂, 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**

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Oxygen and wound healing


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


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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 hypoxia 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.

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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
oxygen to promote wound healing began in the 1960s with administration of systemic hyperbaric O₂ (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, O₂ concentration, frequency and duration of administration) for systemic hyperbaric O₂ 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% O₂ 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 pO₂ 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 O₂ may result in oxidative stress and genotoxicity [20]. There is no question that exposure to pure O₂ 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 O₂ under normobaric conditions [21] lead to question the use of pure O₂ under pressure for wound therapy. Furthermore, encouraging outcome obtained from the use of topical O₂ alone [19] warranted a more detailed investigation testing the efficacy of topical O₂ treatment under controlled conditions. Such fine-tuning of conditions for O₂ therapy should result in a more cost-effective and efficient care minimizing barotraumas and other risks associated with use of pressurized pure O₂. If proven to be efficient, topical O₂ 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 analgesic, 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 pO2 measurements

Real-time wound-bed pO2 was performed non-invasively using Oxy-Lite (Oxford-Optronix, Oxford, UK) as described by us previously [17,26]. An O2 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 deparafrinized 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
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 x 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 pO2 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 O2 administration accelerates vessel growth [31]. VEGF is a major long-term angiogenic stimulus at the wound site. O2 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 O2 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 O2 dependent. The enzymes prolyl hydroxylase, lysyl hydroxylase and lysyl oxidase all require molecular O2 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 hydroxylase and finally cross-linking between large fibrils is performed by lysyl oxidase. These extracellular cross-linkages are ultimately
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 Michaelis-Menten 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$_2$O$_2$ 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

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**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.

**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

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References


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)

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).

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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The use of topical pressurised oxygen therapy

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.
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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 pO2, 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 pO2.

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.

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.

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

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

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 O2 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
The use of topical pressurised oxygen therapy

Table 2 Interpretation of the evidence

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

Table 3 Topical pressurised oxygen therapy: quick reference guide

<table>
<thead>
<tr>
<th>Category</th>
<th>Statement</th>
<th>Strength</th>
</tr>
</thead>
<tbody>
<tr>
<td>Product description</td>
<td>Topical pressurised oxygen therapy is an adjunctive modality/device designed to support wound healing.</td>
<td>Level Iia</td>
</tr>
<tr>
<td></td>
<td>Topical pressurised oxygen therapy delivers humidified oxygen to the wound bed at cyclical pressures above atmospheric pressure.</td>
<td>Level Iia</td>
</tr>
<tr>
<td></td>
<td>Topical pressurised oxygen therapy delivers oxygen into the wound bed, penetrating into the tissue approximately 2 mm deep.</td>
<td>Level Iib</td>
</tr>
<tr>
<td>Patient selection</td>
<td>Topical pressurised oxygen therapy is indicated for the treatment of chronic wounds such as diabetic/neuropathic foot ulcers, venous stasis ulcers and pressure ulcers.</td>
<td>Level Iia</td>
</tr>
<tr>
<td></td>
<td>Topical pressurised oxygen therapy is contraindicated if the patient has an untreated acute DVT or acute thrombophlebitis.</td>
<td>Level IV</td>
</tr>
<tr>
<td>Patient preparation</td>
<td>The presence of necrotic tissue must be minimised in the wound bed prior to the initiation of therapy.</td>
<td>Level III</td>
</tr>
<tr>
<td></td>
<td>The cause(s) of trauma and cofactors that may interfere with healing of the wound must be removed prior to the initiation of therapy.</td>
<td>Level IV</td>
</tr>
<tr>
<td></td>
<td>Client and caregiver concerns must be addressed prior to the initiation of therapy.</td>
<td>Level IV</td>
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<td></td>
<td>Topical dressings post-therapy must meet the needs of the wound in terms of debridement and bacterial and moisture balance.</td>
<td>Level IV</td>
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<td></td>
<td>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.</td>
<td>Level IV</td>
</tr>
<tr>
<td>Application principles</td>
<td>The frequency and duration of therapy is dependent on wound aetiology, wound response and patient tolerance.</td>
<td>Level IV</td>
</tr>
<tr>
<td>Evaluating therapy</td>
<td>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.</td>
<td>Level III</td>
</tr>
<tr>
<td></td>
<td>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.</td>
<td>Level IV</td>
</tr>
<tr>
<td>Expected outcomes</td>
<td>Increased wound oxygenation, through the application of topical pressurised oxygen, results in increased collagen deposition and tensile strength.</td>
<td>Level Iia</td>
</tr>
<tr>
<td></td>
<td>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.</td>
<td>Level Iia</td>
</tr>
<tr>
<td></td>
<td>A lower recurrence rate may be expected in venous leg ulcers and diabetic foot ulcers following topical pressurised oxygen therapy.</td>
<td>Level III</td>
</tr>
<tr>
<td></td>
<td>Topical pressurised oxygen therapy may reduce wound-related pain in venous leg ulcers.</td>
<td>Level III</td>
</tr>
<tr>
<td>Resources</td>
<td>Education needs to be provided to patients, caregivers and healthcare providers regarding the purpose and process of using topical pressurised oxygen therapy.</td>
<td>Level IV</td>
</tr>
<tr>
<td></td>
<td>Preliminary studies have shown that topical pressurised oxygen therapy has the potential for cost savings.</td>
<td>Level IV</td>
</tr>
<tr>
<td>Safety and precautions</td>
<td>Protocols for oxygen safety must be followed when therapy is in use.</td>
<td>Level IV</td>
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DVT, deep venous thrombosis.
The use of topical pressurised oxygen therapy

adequate supply of oxygenated blood. Studies show that tissues must have a pO2 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 pO2, 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).

**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).
The use of topical pressurised oxygen therapy

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
The use of topical pressurised oxygen therapy

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).

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

| 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.
The use of topical pressurised oxygen therapy is 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 pO₂ 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

- 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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