Continuous diffusion of oxygen improves diabetic foot ulcer healing when compared with a placebo control: a randomised, double-blind, multicentre study

Objective: The aim of this study was to assess whether continuous diffusion of oxygen improves healing in people receiving treatment for diabetic foot ulcers (DFU).
Method: A double-blind, placebo control randomised study to receive either active continuous diffusion of oxygen (CDO) therapy using an active CDO device, or a fully operational placebo device without delivering oxygen. Patients were followed until closure or 12 weeks. Patients, caretakers, treating physicians and independent evaluators were blinded to the study arm. All patients received identical offloading, debridement, dressings and follow-up.
Results: We enrolled 146 people with DFUs (77% male, aged 56.3±12.4 years). A significantly higher proportion (195%) of DFUs healed in the CDO arm compared with placebo (32.4% versus 16.7%, p=0.033). The time to 50% DFU closure was significantly shorter in patients that received CDO therapy (mean 18.4 versus 28.9 days, p=0.001). There were no differences in overall adverse events (p=0.66) or ulcer-related adverse events (p=0.30) in the active and placebo treatment groups. The relative performance of active CDO over placebo became greater when used in larger wounds (273%), in more chronic wounds (334%) and in weight bearing wounds (465%).
Conclusion: The results of this study demonstrate that CDO leads to higher proportion of healed DFUs (p=0.033) and a faster time to closure compared with placebo in people with DFUs (p=0.015). Relative performance did not vary significantly with wound size (p=0.80), but revealed better relative performance in more chronic wounds (p=0.008) and in weight-bearing wounds (p=0.003).
Declaration of interest: MQN is a full-time employee of EO2 Concepts. DGA and LAL are a member of the scientific advisory board of EO2 concepts. The other authors have no conflict of interest to declare.

• closure rate • continuous diffusion of oxygen • diabetic foot ulcer • moist wound therapy • tissue oxygenation • wound healing

Diabetic foot ulcers (DFUs) and their resultant complications constitute a silent, sinister burden for patients and society at large.[1–4] Approximately half of DFUs become infected.[5] Once this occurs, some 20–30% of patients will receive some form of amputation.[2,6] Following amputation, five year survival is worse than most forms of cancer.[7–8] Furthermore, the direct costs associated with care of the diabetic lower extremity are greater than the five most expensive cancers in the US alone.[9] Treating the wounds to closure as rapidly and safely as possible, therefore, is a logical strategy to reduce morbidity while dramatically improving the cost-effectiveness of the treatment.

Oxygen has been shown to be an essential component in multiple mechanisms of action required for wound healing.[10–12] Depressed levels of oxygen have been shown to be a rate-limiting step in these mechanisms. Conversely, increasing oxygen levels has been shown to result in increased, and often proportional, levels of activity in these mechanisms of action. Aside from general cell metabolism and energy production, these mechanisms of action, and corresponding rates of action, affected by oxygen levels in the tissue include: cell proliferation and re-epithelialisation,[13–14] collagen synthesis and tensile strength,[15–17] angiogenesis,[18–19] antibacterial activity through respiratory burst[20–22] and growth factor signalling transduction.[23–24] The damaged tissue in chronic wounds has increased oxygen demands and can achieve improved healing through enhancement of local oxygen availability with oxygen therapy.[10,12,22] The use of occlusive dressings blocks or limits the amount of oxygen available to the wound from the surrounding atmosphere, thus increasing the need for supplemental oxygen. This is further compounded for patients with chronic wounds who often have local,

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compromised blood flow to the wound, thus further limiting tissue oxygenation and impairing healing.[25]

Enhancing tissue oxygenation can be achieved using different therapies.[26] Traditionally, hyperbaric oxygen therapy has been used to achieve supersaturated levels of oxygen in the blood stream and tissues through high pressures and concentrations of inspired oxygen. However, this therapy is intermittent (90-minute exposures, 3–5 times per week), relies on circulation (which may be impaired) to bring the oxygen to the damaged tissue and requires substantial time for the patient in terms of travel and preparation time to receive daily therapy. Topical oxygen therapy is a newer modality in which the affected tissue is placed within a chamber or bag and exposed to high concentrations of oxygen (~93% oxygen). However, this therapy is also intermittent (typically 90-minute exposures once a day), requires a dressing change and the subject must remain immobile during the treatment. However, topical oxygen can be applied in a wider variety of settings, including the patient's home. The newest therapy, continuous diffusion of oxygen or continuously diffused oxygen (CDO), removes the above-mentioned limitations of being intermittent and immobilising the patient during treatment. CDO uses pure, humidified oxygen to continuously treat a wound by supplying oxygen directly to the affected tissue within a moist wound therapy (MWT) dressing. This allows for sustained delivery of oxygen to the tissue (24 hours a day, seven days a week), full patient mobility during treatment and application of the therapy in virtually any setting. Devices which supply CDO therapy are lightweight, silent, solid-state, and come in rechargeable or disposable versions.[26]

This study focuses on an Food and Drug Administration-cleared device which supplies CDO therapy, the TransCu O2 System (EO2 Concepts, San Antonio, TX). The device uses fuel cell technology to continuously generate pure (>99.9%), humidified oxygen at flow rates of 3–15ml/hour and delivers it directly to the wound bed environment within the MWT dressing system via tubing. CDO therapy can be simply described as MWT plus humidified oxygen. This CDO device is the only system to employ sensors that monitor and control the flow rate of oxygen being delivered and the pressure within the wound bed. The oxygen control system compensates for environmental fluctuations that can affect oxygen output, thus ensuring consistent oxygen delivery. The pressure control system ensures that there is no blockage of oxygen flow and, perhaps more importantly, that the oxygen pressure in the wound bed does not exceed capillary collapse pressure by limiting the maximum sustained gauge pressure in the wound bed to 20mmHg. Excessive localised pressures could collapse capillaries and impair delivery of blood and nutrients to the affected tissues during wound repair. The unit continuously monitors and controls for these variables, and warns the patient/physician if the flow of oxygen is impaired or if the pressure in the wound bed is too high.

Previous studies have demonstrated the efficacy of CDO using various devices. A retrospective analysis on the impact of CDO in chronic toe ulcer healing for 20 patients showed an overall success rate (full closure) of 74% on wounds that were unresponsive to other therapies.[27] This report highlighted a chief benefit being that of high patient compliance (95%), which was attributed to the device's ease of use, the noticeability of improvement within a short period of time, and the reduction of pain, which has also been reported elsewhere.[28] Another retrospective analysis of 25 patients in a Veteran's Healthcare Administration environment showed 68% full closure, both as a stand alone and adjunctive therapy.[29] In this report it was found that CDO improved wound healing potential, including in wounds receiving advanced tissue/skin substitute applications. A prospective, randomised clinical trial of CDO versus MWT followed 17 patients (nine CDO, eight MWT) for four weeks and found significant differences in wound volume reduction.[30] The CDO group had an average volume reduction of 87%, whereas the MWT group had an average volume reduction of 46% ($p<0.05$). Significant differences in the healing rate of CDO as compared with MWT were recently demonstrated in a prospective, randomised pilot clinical trial with nine patients receiving MWT and 9 receiving CDO.[31] The study focused on smaller DFUs (approx. 1.5cm^2), University of Texas (UT) Grade I–III , over an 8 week period. CDO was shown to close 90% of the wounds by the end of the study, whereas the MWT group experienced 30% closure. The authors also noted significantly faster wound closure rates in the CDO arm and more noticeable differences from CDO in the more advanced ulcers (Grade II and III).

In a double-blind preclinical study using a placebo as the control arm, all wounds received MWT dressings and a CDO device; significant results were found for both the rate of re-epithelialisation and amount of full closure achieved.[13] Full closure was 57% in the active CDO arm and 25% in the placebo arm ($p=0.008$), for a relative performance of 228%. The rate of re-epithelialisation was 65% faster in the active arm as compared with the placebo arm ($p=0.006$). The authors also noted that histologically the repaired tissue showed more advanced wound remodelling and organised collagen in the active CDO arm. Similarly, a non-placebo blinded preclinical trial showed significant results with each animal as its own control. The rate of re-epithelialisation was increased in the CDO arm by 156% relative to the control arm ($p=0.01$).[32]

Pain reduction associated with CDO therapy has also been reported. For a patient who served as her own control during CDO therapy treatment, her pain levels were reported as high as 8/10 on a visual analogue score (VAS), with pain medications taken as

needed, during the five-month duration of the ulcer before CDO therapy.[33] After 20 days of CDO therapy, the patient reported a pain level of 2/10 and was no longer taking pain medications. At this time, CDO therapy was temporarily discontinued since the patient was leaving town for a holiday. The patient returned to the clinic six days later with a pain level of 10/10 and reported difficulty sleeping. CDO therapy was reapplied and, within three days, her pain level was controlled (VAS 2/10) and she ceased taking narcotics. In an uncontrolled, non-randomised study of 10 patients with venous ulcers, CDO therapy was reported to significantly ($p<0.009$) reduce pain in a six week period.[34] The corresponding mean reduction in wound size was 58.9%. In a case series review, four patients with severe, very painful wounds were successfully closed and the pain was significantly reduced in all cases.[35] Similarly, a six patient case review of CDO in patients with diabetes and chronic lower extremity wounds reported significant pain reduction.[36]

This report focuses on the intent-to-treat analysis of the effect of CDO and is a continuation of the reported per protocol results.[37] Additional data and results are presented here that were not available at the time of that publication since the study was still ongoing for patients who were not in the first 50 to complete in each group, as well as patients who were in the follow-up phase. Comparisons are made to the per protocol analysis throughout. The primary outcome is complete wound closure by 12 weeks. Secondary outcomes of rate of wound closure and follow-up durability are presented.

Materials and methods

Study design, primary and secondary outcomes

This is a planned analysis of a 12-week, randomised, fully blinded, placebo-controlled, parallel group clinical trial evaluating the use of the CDO device for DFUs (ClinicalTrials.gov Identifier NCT01645891). The CDO device used was the TransCu O2 System. The study was approved by the Schulman Associates Institutional Review Board (Cincinnati, OH, IRB No. 201202439). Randomisation lists were made by the statistician for each clinical site in blocks of size four with SAS. Devices were labelled by the statistician before shipping to the sites. The sites assigned devices to patients sequentially at randomisation. Both arms received identical treatment (device, dressings, etc.) and the devices were functional in both arms with the exception that the oxygen did not flow to the ulcer in the placebo arm (Fig 1). All devices were functional in that they produced oxygen and displayed the oxygen flow rate. This had the effect that the devices appeared identical, including battery drain and oxygen flow display. The only difference was that the placebo devices did not have any oxygen flowing out of the oxygen supply port. Since the oxygen flow rate (3ml/hour) is low enough that it cannot be felt by the subjects or physicians, the devices all appeared identical. Similarly, the dressings and offloading boots in each arm were limited and identical. The result was that the patients, doctors, evaluators, sponsor and statistician were all fully-blinded to the treatment arms until the data had been collected and verified, thereby eliminating the placebo effect.

Before assignment of a device, all patients were subjected to a run-in period during which they received standard of care dressings, debridement and off-loading to ensure that the wounds were indeed chronic in nature (Fig 2). There were two inclusion criteria essential to the design of the study to ensure that only chronic wounds were being included: initial or baseline wound size and initial or run-in rate of wound closure. These were defined as:

Fig 1. Study designed with placebo to eliminate bias. All factors were set to be identical in both treatment arms, including functional devices, dressings and offloading. The only difference was that the oxygen produced by the device in the placebo arm did not flow out the tubing to the wound and dressing.

Active arm	Placebo arm
TransCu O_2 system preset at 3ml/hour (oxygen flowing to wound 24/7)	TransCu O_2 system preset at 3ml/hour (no oxygen flowing out)
ExtraSorb Foam Dressing (Derma Sciences) Tegaderm Occlusive Layer (3M) DH OffLoading Walker Boot (Össur) (optional) Maxorb Calcium Alginate (Medline) for excessive exudate control	

Fig 2. Study flow chart

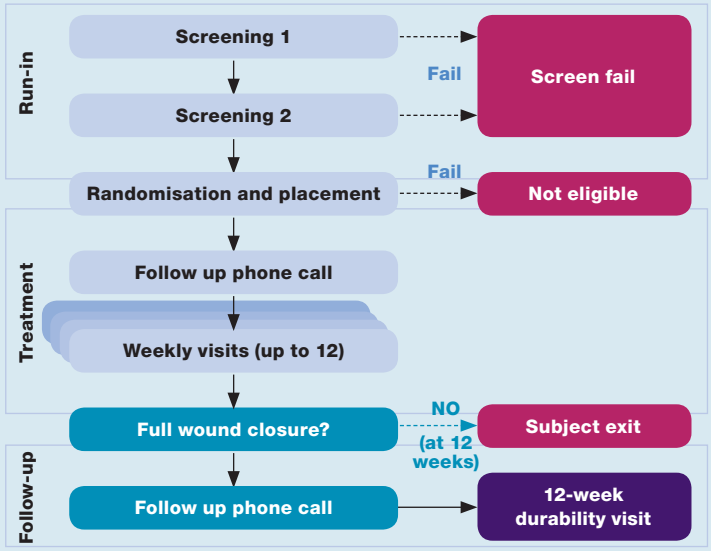
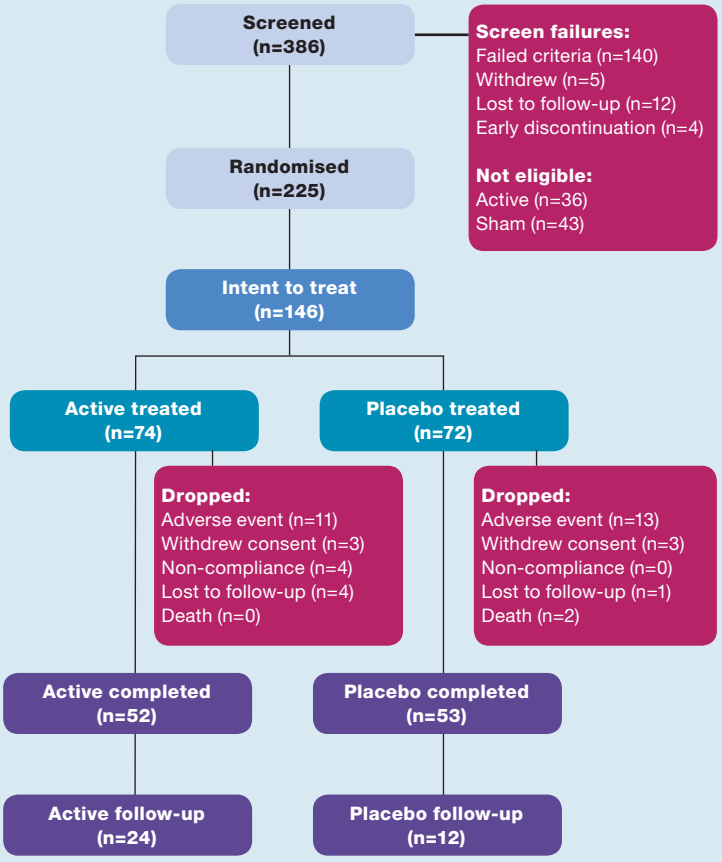


Fig 3. Consort diagram



- Baseline DFU size: the ulcer area as determined by independently-verified digital planimetric analysis at the randomisation visit.
- Run-in ulcer closure rate: the percentage of ulcer closure (percentage wound area reduction, or PWAR) during the run-in period before the placement of the device. All subjects received MWT during the run-in period.

The intent was to find a balance between a short run-in period and robust screening criteria to help ensure that non-chronic wounds were not included in the study. Since the PWAR assessment relied on independently-verified planimetric analysis of wound photos, some subjects were initially placed on a device at the conclusion of the run-in period and subsequently found to be not eligible for failing study inclusion/exclusion criteria. These subjects were removed as not eligible and are not included in the treated number of subjects shown on the consort diagram (Fig 3).

The primary efficacy outcome was complete wound closure, defined as complete re-epithelialisation with no drainage as assessed by the treating clinician and confirmed by a blinded observer. Secondary outcomes of rate of ulcer closure, adverse events and follow-up durability are presented. The effects of baseline ulcer size and run-in ulcer closure rate, which are measures ulcer chronicity are reported. The impact of race/ethnicity and ulcer location (weight-bearing or not) are also discussed.

Screening, randomisation and treatment

Eligible subjects were those who were confirmed to meet all inclusion and none of the exclusion criteria (Table 1). Those with a DFU present for a minimum of 30 days, yet not more than a year, were eligible for enrolment, between 30 and 90 years of age, with sizes ranging from 1.5cm^2 to 10cm^2, as measured by independently-verified planimetric analysis from photos taken after ulcer debridement. Subjects were recruited from a total of 34 sites in the continental US. After initial screening for eligibility and obtaining informed consent, a patient history and baseline assessment were obtained by the study clinician. Variables assessed included: ankle-brachial index (ABI), ulcer duration, location and size, patient age, race, gender, and glycated haemoglobin (HbA1c). All ulcers were classified according to the University of Texas classification for DFUs by a wound specialist, based on clinical and laboratory data.[38] Only UT Class 1A ulcers were included. Refer to Table 1 for a complete list of inclusion and exclusion criteria.

All ulcers were surgically debrided to a bleeding base as necessary; the number of debridements was not limited but usually debridements were performed once a week. All subjects received a standard regimen consisting of wound cleansing, MWT, off-loading and, as appropriate, aggressive debridement. As shown in Fig 1, the dressings in both arms were identical and restricted to a single foam (Xtrasorb Non-Adhesive Foam, Derma Sciences) covered by an occlusive barrier (Tegaderm, 3M Medical) to eliminate dressing variability. Optionally, a calcium alginate (Maxorb, Medline) could be used for control of excessive exudate. DH Offloading Walker boots (Össur) were used for off-loading.

Subjects were randomised to either the Treatment arm with an active CDO device (CDO arm) or the control arm using a placebo CDO device (placebo arm). Subject recruitment occurred from June 2012 to November 2016. The last subject exited the follow-up phase of the study in April 2017.

All patients were followed for the treatment phase of 12 weeks, or until the wound closed, whichever event occurred first. During the treatment phase, weekly visits were made and included ulcer assessment, debridement, digital photograph for DFU size determination via planimetric analysis, documentation of adverse events and a dressing change with reapplication of the study device. Between visits, the study device was used continuously (24 hours per day, seven days per week) and dressings were changed as needed by the subjects themselves or a relative/friend for the vast majority of subjects (>99%), demonstrating the ease of application and use by patients in the general population.

Table 1. Inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
30–90 years old at the time of consent	<30 or >90 years old at the time of consent
Type 1 or type 2 diabetes mellitus with a non-healing, full-thickness, University of Texas Classification of Diabetic Foot Ulcers Class IA diabetic foot ulcers	Subjects with target ulcers with a duration <4 weeks or >52 weeks
An ulcer with a duration of at least 4 weeks, but not greater than 52 weeks at time of Screening 1	Ulcers measuring <1.5cm^2 or >10cm^2 in area after debridement at the time of randomisation, as measured using digital photography and computerised planimetric analysis by the Centralized Wound Measuring Center (CWMC)
An index ulcer measuring between 1.5–10cm^2 in area after debridement at time of randomisation, as measured using digital photography and computerised planimetric analysis by the CWMC	Ulcer decreased in area by >30 % during either 1 week screening period (screening 1–screening 2 or screening 2–randomisation) or >50 % during the two-week screening period, as measured using digital photography and CWMC
Diabetic foot ulcer(s) at or below the malleoli	Evidence of gangrene on any part of affected limb
Adequate arterial perfusion defined as one or more of the following: ● Transcutaneous oxygen measurements of the dorsum of the foot >30mmHg with a skin perfusion pressure >30mmHg ● An ankle/brachial index (ABI) >0.7, with documented confirmation of adequate arterial perfusion ● A Doppler waveform consistent with adequate flow in the foot (biphasic or triphasic waveforms) at screening ● Absolute toe pressure of >30mmHg	Active Charcot's foot on the study limb
	Subjects scheduled to undergo vascular surgery, angioplasty or thrombolysis at the time of enrolment
	Active infection at the time of screening
	A target ulcer which has exposed tendons, ligaments, muscle, or bone
	Active malignancy, excluding non-melanoma skin cancer
Subject and/or caregiver must be able and willing to learn and perform the duties of dressing changes	A history of malignancy on study limb
Subjects who need off-loading devices are able and willing to comply with standardised off-loading regimen (such as a fixed ankle walker)	Subjects in whom oral, or IV antibiotic/antimicrobial agents or medications have been used within 2 days (48 hours) of baseline
	Receiving or has received radiation or chemotherapy within three months of randomisation
	Subjects who have received growth factor therapy (e.g., autologous platelet-rich plasma gel, becaplermin, bilayered cell therapy, dermal substitute, extracellular matrix) within two weeks of screening
	Subjects who are pregnant at the time of screening, are undergoing active renal dialysis, have a known immune insufficiency, excluding diabetes mellitus, a history of peripheral vascular repair within 14 days of screening, a current deep vein thrombosis (DVT), with ulcers due to Raynaud's disease, an ulcer due to acute thrombophlebitis
	Inadequate perfusion to support healing
	Necrotic wounds covered with eschar or slough or wounds with fistulae or deep sinus tracts of unknown depth
	Subjects who are receiving palliative care
	A HbA1c >12% (uncontrolled hyperglycaemia)
	A known aetiology of: malignancy, burn, collagen vascular disease, sickle cell, vasculopathy, or pyoderma gangrenosum
	A documented history of alcohol or substance abuse within six months of screening
	Enrolled or who have participated, within 30 days of Screening 1, in another investigational device, drug or biological trial that may interfere with study results
	A known allergy to dressing materials, including occlusive dressings and the adhesives on such dressings

To evaluate whether there was an effect of DFU size on the primary outcome of ulcer closure, the effect on the primary outcome was analysed by DFU size using equal numbers of subjects in quartiles, from the smallest 25% to the largest 25% of DFUs. This allowed for at least 25 subjects in each group. To evaluate whether there was an effect of run-in closure rate on the primary outcome of closure, results were reported by analysing the results of subjects who experienced closure less than two lower rates of PWAR, each corresponding to more chronic DFUs. The lowest rate analysed was chosen to match that established by Lavery et al. as defining a chronic wound which would benefit from advanced intervention: <60% PWAR in four weeks.[39] This in turn corresponds to <30% in two weeks, the run-in period used in this study. The intermediate rate of <40% PWAR in two weeks was also analysed. Since each two-week period analysed had a 10% change from the original 50%, the corresponding one-week period rate of change was 5%. Therefore, the

analysis included wounds that experienced <25% PWAR in either week of the first two weeks or <40% PWAR in the first two weeks (referred to as a run-in wound closure rate of 25%/40% PWAR), as well as < 20% PWAR in either week of the first two weeks or < 30% PWAR in the first two weeks (referred to as a run-in wound closure rate of 20%/30% PWAR). We defined relative performance as the ratio of the proportion of subjects in the CDO arm reaching full closure divided by the proportion of subjects in the placebo arm reaching full closure (relative risk), expressed as a percentage.

Statistical methods

This study was planned to follow a group sequential design with one interim analysis at the midpoint and one at the endpoint of the study with O'Brien-Fleming stopping bounds. We assumed that 82.4% of treated and 45.5% of controls would experience wound closure; with two-sided testing, an interim analysis when 50% and 100% of subjects complete the treatment phase, O'Brien-Fleming stopping bounds, and an overall significance level of 5%, then this study would achieve 90% power with 41 subjects per treatment arm.

Table 2. Baseline (enrolment) characteristics of all treated subjects

Characteristics	CDO (n=74)	Placebo (n=72)	Total (n=146)	p-value
Age ± SD	56.1±10.1	56.6±14.4	56.3±12.4	0.84
Female (%)	15 (20.3)	18 (25.0)	33 (22.6)	0.63
Male (%)	59 (79.7)	54 (75.0)	113 (77.4)	
Ethnicity (%)				
Black	13 (17.6)	12 (16.7)	25 (17.1)	0.73
Hispanic	26 (35.1)	29 (40.3)	55 (37.7)	
White	34 (45.9)	31 (43.1)	65 (44.5)	
Other	1 (1.4)	0 (0.0)	1 (0.7)	
Wound area, cm^2 ± SD	3.54±1.68	3.89±2.02	3.71±1.85	0.27
Wound age, days ± SD	131.6±89.2	143.8±97.7	137.6±93.4	0.43
HbA1c ±SD	8.4 ± 1.6	8.3 ± 2.0	8.3±1.8	0.88
ABI±SD	1.05±0.14	1.02±0.15	1.03±0.15	0.25
Weight bearing (%)	59 (79.7)	53 (73.6)	112 (76.7)	0.50
No pain (%)	32 (43.8)	26 (36.6)	58 (40.3)	0.48
Pain (%)	41 (56.2)	45 (63.4)	86 (59.7)	

SD—standard deviation; ABI—ankle-brachial index; CDO—continuously diffused oxygen

The study failed to cross the boundary at midpoint; at the interim analysis of primary outcome based on n=42 subjects (n=21 in each arm) 52.4% of CDO and 38.1% of placebo subjects experienced wound closure (p=0.54). In a conditional power calculation, we concluded that if the efficacy specified in the protocol was experienced in the remainder of the study, then the total sample size required to reach 90% power would be 100 or n=50 per arm.

Subjects who failed to meet eligibility criteria were excluded. Continuously distributed outcomes were summarised with the sample size, mean, and standard deviation, and categorical outcomes were summarised with frequencies and percentages. When contrasting treatment arms with regard to binary outcomes, we report the relative risk and its 95% confidence interval (CI); in this report the relative rRisk is also referred to as relative performance. At baseline, treatment arms were contrasted on the mean of continuously distributed outcomes with t-tests and on proportions with Fisher's Exact test. The significance of relative performance was assessed with a generalised estimating equation (GEE) Poisson model with a log-link. The significance of variation in the risk of adverse events with treatment arm was assessed with Fisher's Exact test. We used R and SAS for all analyses and graphics. All statistical tests were two-sided with a significance level of 5%. Corrections for multiple comparisons were not applied.

Results

During the course of the study, 386 subjects were screened, and 146 subjects entered the treatment phase and were allowed to complete the study (Fig 3). The greatest reason for the 140 screen fails was wound size (48.6%) and the second highest reason was the PWAR (32.9%). However, for the 79 subjects determined to be not eligible, the run-in closure rate became the highest reason for exclusion (59.5%), with wound size dropping to the second highest reason (34.2%). At baseline the two treatment arms were similar with regard to age, ethnicity, gender, DFU size, age of DFU, glycated hemoglobin, ABI, DFU location (weight bearing or not) and patients experiencing no pain in the ulcer (Table 2). Analysis of DFU size (cm^2) at baseline (Fig 4) shows that it did not vary significantly by treatment arm (p=0.56) or DFU closure (p=0.089). The DFUs were 4.5 months old on average, and most were on the plantar, weight-bearing aspect of the foot (76.7%).

Table 3. Primary outcome of full wound closure for all subjects who entered the treatment phase

Analysis	CDO	Placebo	Relative risk	95% CI	p-value
ITT – all treated	32.4% (74)	16.7% (72)	1.95	(1.05 to 3.59)	0.033
ITT – all completed	46.2% (52)	22.6% (53)	2.04	(1.14 to 3.63)	0.016
Per protocol	46.0% (50)	22.0% (50)	2.09	(1.15 to 3.82)	0.016

CDO—continuously diffused oxygen; CI—confidence interval; ITT—intention to treat; sample sizes are indicated in parentheses; analysed by those subjects who dropped or completed (ITT–all treated), completed the study (ITT–all completed), or were in the first 50 subjects in each arm to complete the study (per protocol)

Fig 4. Wound size at baseline by treatment arm and wound closure in all completed subjects, continuously diffused oxygen (CDO; n=52) and placebo (n=53). Wound size at baseline did not vary significantly with treatment (p=0.56) or wound closure (p=0.089)

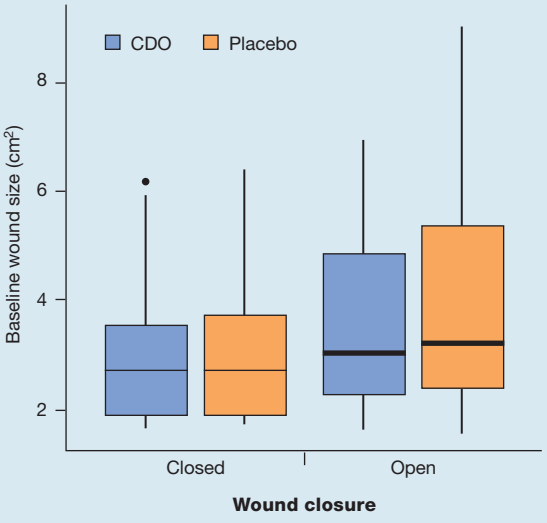
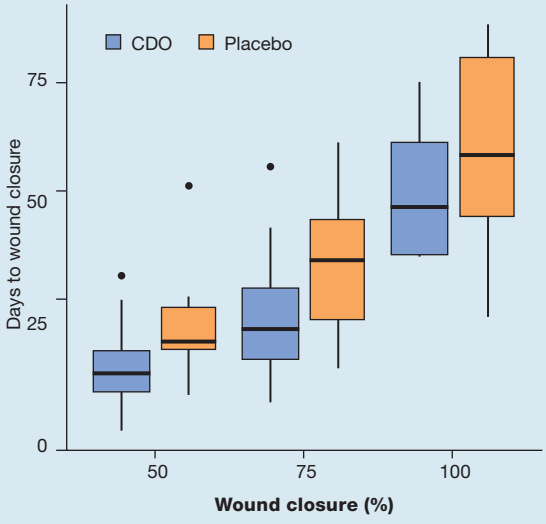


Fig 5. Days to wound closure by treatment arm and wound closure (%) among all treated subjects (n=146). Days to wound closure significantly decreased in the continuously diffused oxygen (CDO) arm relative to placebo (p=0.015)



The use of CDO resulted in significant improvements in the primary outcome of complete wound closure at 12 weeks (Table 3). In the ITT analysis of all treated subjects (including drop-outs) 32.4% CDO and 16.7% placebo obtained full closure (p=0.033) and the relative risk, or relative performance, showed that the CDO arm performed 195% better than the placebo arm, similar to the results for ITT subjects who completed the study (204% relative performance, 46.2% CDO, 22.6% placebo, p=0.016) and to the per protocol analysis (209% relative performance).[37]

The time to ulcer closure was significantly shorter in patients who received CDO therapy. For all treated subjects, including those who dropped and did not complete the study (n=146), days to closure increased with wound closure in both arms (Fig 5); days to wound closure decreased significantly in the CDO arm relative to placebo (p=0.015). The time to 50% DFU closure was significantly shorter in patients that received CDO therapy (mean 18.4 versus 28.9 days, p=0.001).

DFU closure (%) by treatment arm and run-in wound closure rate (PWAR) for all completed subjects is summarised in Table 4. There was increasing significant beneficial effect of the CDO arm at 25%/40% PWAR (p=0.017) and 20%/30% PWAR (p=0.008). Closure in

Table 4. Wound closure (%) by treatment arm and run-in wound closure rate (PWAR) for all completed subjects

PWAR	CDO	Placebo	Relative risk	95% CI	p-value
30%/50%	46.2% (52)	22.6% (53)	2.04	(1.14 to 3.63)	0.016
25%/40%	43.8% (48)	18.6% (43)	2.35	(1.16 to 4.75)	0.017
20%/30%	43.9% (41)	13.2% (38)	3.34	(1.37 to 8.1)	0.008

CI—confidence interval; CDO—continuously diffused oxygen; PWAR—percentage wound area reduction; sample sizes are indicated in parentheses. The lower PWAR numbers are indicative of more chronic wounds. The results indicate that the Active CDO Arm performs better as compared with MWT alone as the wounds become more chronic

Table 5. Wound closure (%) by treatment arm and quartile size range for all completed subjects (n=105)

Quartile size range (cm^2)	CDO (n=52)	Placebo (n=53)	Relative risk	95% CI	p-value
1.5–2.15	58.33% (12)	35.71% (14)	1.63	(0.7 to 3.82)	0.258
2.15–3.0	46.67% (15)	25.00% (12)	1.87	(0.61 to 5.72)	0.274
3.0–4.9	42.86% (14)	16.67% (12)	2.57	(0.63 to 10.45)	0.187
>4.9	36.36% (11)	13.33% (15)	2.73	(0.6 to 12.33)	0.192

CI—confidence interval; CDO—continuously diffused oxygen; sample sizes for each arm in each quartile are indicated in parentheses

Table 6. Effect of DFU location, weight-bearing or non-weight-bearing, on outcomes for all treated subjects (n=146)

a) Number of weight-bearing DFUs					
All treated	**CDO**	**Placebo**	**Relative risk**	**95% CI**	**p-value**
% Weight bearing	79.73% (74)	73.61% (72)	1.08	(0.42, 1.39)	0.5

b) Wound closure for weight-bearing and non-weight-bearing DFUs					
All treated	**CDO**	**Placebo**	**Relative risk**	**95% CI**	**p-value**
Weight bearing	33.9% (59)	7.55% (53)	4.49	(1.64, 12.3)	0.003
Non-weight bearing	26.67% (15)	42.11% (19)	0.63	(0.24, 1.71)	0.37

DFU—diabetic foot ulcer; CDO—continuously diffused oxygen CI—confidence interval; a) the percentage of subjects in each arm that had ulcers in weight-bearing locations; b) the percentage of ulcers that closed in the weight-bearing group and non-weight-bearing group, respectively. Sample sizes for each arm in each grouping are indicated in parentheses

Table 7. Effect of DFU location, weight-bearing or non-weight-bearing, on outcomes for all completed subjects (n=105)

a) Number of weight bearing DFUs by treatment arm					
All Treated	**CDO**	**Placebo**	**Relative risk**	**95% CI**	**p-value**
% Weight-bearing	82.7% (52)	75.5% (53)	1.1	(0.33 to 1.51)	0.5

b) Wound closure for weight bearing and non-weight bearing DFUs and treatment arm					
All Treated	**CDO**	**Placebo**	**Relative risk**	**95% CI**	**p-value**
Weight-bearing	46.5% (43)	10.0% (40)	4.65	(1.74 to 12.43)	0.002
Non-weight-bearing	44.4% (9)	61.5% (13)	0.72	(0.31 to 1.69)	0.45

DFU—diabetic foot ulcer; CDO—continuously diffused oxygen; CI—confidence interval; a) the percentage of subjects in each arm that had ulcers in weight-bearing locations; b) the percentage of ulcers that closed in the weight-bearing group and non-weight-bearing group, respectively. Sample sizes for each arm in each grouping are indicated in parentheses

Table 8. Durability of DFU at 12-week follow-up

	CDO	**Placebo**	**Relative risk**	**95% CI**	**p-value**
Closed at follow-up	87.5% (24)	90.0% (10)	0.97	(0.75 to 1.26)	0.83

DFU—diabetic foot ulcer; CDO—continuously diffused oxygen; CI—confidence interval; the percentage of wounds remaining closed at the 12-week follow-up visit is shown for each arm. Sample sizes for each arm completing the follow-up phase are indicated in parentheses

Table 9. Comparison of median time-to-closure between dropped subjects and all treated

	Days to 50% closure			**Days to 75% closure**		
	CDO	**Placebo**	**p-value**	**CDO**	**Placebo**	**p-value**
All treated	18.4 (40)	28.9 (37)	0.001	27.8 (34)	40.0 (25)	0.04
Dropped	9.7 (3)	20.7 (4)		27.8 (2)	39.6 (2)	

CDO—continuously diffused oxygen. The median number of days to reach 50% and 75% wound closure is shown along with the number of subjects in parentheses for all treated subjects (all treated) as well as the subgroup which dropped from the study (dropped). Sample sizes for each arm in each grouping are indicated in parentheses

the CDO arm was relatively insensitive to reducing the run-in DFU closure rate (values ranged from 46.2% to 43.8% full closure), whereas the placebo arm experienced a decrease from 22.6% full closure to 13.2% full closure, corresponding to a 42% reduction in efficacy as the DFUs become more chronic. This resulted in an overall increase in relative performance of the CDO versus placebo from 204% to 334% as the wounds become more difficult to heal.

Table 5 summarises DFU closure (%) by treatment arm and quartile baseline wound size range for all completed subjects (n=105). The CDO arm shows a corresponding increase in performance relative to the placebo arm as the wound size increases.

The effect of ulcer location (weight-bearing or not) is shown in Tables 6 and 7 for all treated subjects and those who completed the study, respectively. For all subjects who entered the treatment phase (Table 6), the percentage of ulcers which were in weight-bearing locations were similar between the two arms (79.7% CDO, 73.6% in placebo, p=0.50). However, the percentage of ulcers that closed were significantly different between the arms for weight-bearing locations: 33.9% closed in the CDO arm as compared with 7.6% in the placebo arm (p=0.003). For non-weight bearing DFU locations, more DFUs closed in the placebo arm, yet the differences were non-significant (p=0.37). Similar results are shown for all subjects who completed the treatment phase (Table 7), with no significant difference between the two arms for percentage of weight-bearing DFUs completing the treatment phase (p=0.50) or those experiencing closure in the non-weight-bearing group (p=0.45). The differences were significant for the DFUs in the weight-bearing group, with 46.5% of DFUs in the CDO arm closing versus 10.0% of the placebo arm (p=0.002). The relative performance of CDO versus placebo for weight-bearing DFUs was 465%.

The effect of oxygen on DFU durability was measured by following subjects whose ulcers closed fully and entered the follow-up phase with an assessment 12 weeks after closure (Fig 2). It was documented whether the DFU remained closed or reopened. No special requirements were placed on patients, such as the requirement for offloading, during this period. We lost two subjects to follow-up during this period, both in the placebo arm, one each in the weight-bearing and non-weight-bearing groups, resulting in n=10 for the placebo arm. As can be seen in Table 8, there were no statistical differences between the treatment arms (p=0.83). Of the DFUs that reopened, those in the CDO arm were in weight bearing locations and the one in the placebo arm was in a non-weight-bearing location.

A comparison of the time to DFU closure for subjects that dropped to that for all patients who were treated is shown in Table 9. The time to 50% DFU closure for subjects that dropped is lower in both arms and the time to 75% closure is substantially similar in both arms as compared with the overall results.

Table 10. Primary outcome of full wound closure for all completed subjects (n=105) by their racial/ethnic orientation

Race/ethnicity	CDO	Placebo	Relative risk	95% CI	p-value
Black	57.1% (7)	22.2% (9)	2.57	(0.65 to 10.23)	0.18
Hispanic	55.0% (20)	25.0% (20)	2.20	(0.93 to 5.18)	0.071
White	36.0% (25)	20.8% (24)	1.73	(0.68 to 4.42)	0.25

CDO—continuously diffused oxygen; CI—confidence interval; sample size for each orientation is indicated in parentheses

Table 11. Adverse events

Characteristics	CDO (n=74)	Placebo (n=72)	Total (n=146)	p-value
Adverse events (%)	11 (14.9%)	13 (18.1%)	24 (16.4%)	0.66
Related to study wound (%)	6 (8.1%)	10 (13.9%)	16 (11.0%)	0.30
Requiring hospitalisation (%)	2 (2.7%)	8 (11.1%)	10 (6.8%)	0.054
Gangrene (%)	0 (0.0%)	2 (2.8%)	2 (1.4%)	0.24

CDO—continuously diffused oxygen. The number of subjects who dropped from the study due to an adverse event (and percentage) is shown on the first row. The second row shows number of those adverse events that were related to the study wound. The third row shows the number of study wound adverse events that required hospitalisation. The last row depicts the total number of patients infected in the study wound with gangrene

The median number of days to reach 50% and 75% DFU closure is shown along with the number of subjects in parentheses for all treated subjects (all treated) as well as the subgroup which dropped from the study (dropped). Sample sizes for each arm in each grouping are indicated in parentheses.

The results for the primary outcome by race or ethnicity are shown in Table 10 among those who completed the study (n=105). The distribution of CDO and placebo devices was balanced. For each group, the response for the CDO arm was higher than the placebo arm, although not statistically significant. The relative performance (relative risk) for each ethnic group was similar to those for all completed subjects, ranging from CDO being 173% (among White) to 257% better at closing wounds (among Black), as compared with 204% for all subjects who completed the study.

There were no differences in overall adverse events (p=0.66) or DFU-related adverse events (p=0.30) in the CDO and placebo treatment arms (Table 11). However, about half as many of the adverse events were related to the study wound in the CDO arm as compared with the placebo arm. Of adverse events related to the study DFUs, all were due to infection in both arms. The degree of infection was higher in the placebo arm, as evidenced by the number requiring hospitalisation: four times as many subjects were hospitalised due to infection of the target DFU in the placebo arm compared with the CDO arm. Furthermore, two of the DFUs became infected with gangrene in the placebo arm and none did in the CDO arm.

Discussion

In this study we found a significant treatment effect in the CDO arm for the primary outcome of full ulcer closure for subjects who completed the study, with 46.2% of CDO-treated DFUs closing versus 22.6% in the placebo arm and for all subjects that were randomised in the study: 32.4% CDO and 16.7% placebo. These results support the findings that supplemental oxygen can meet the increased oxygen demands of damaged tissue in chronic wounds and thereby enable improvement in the healing process.[10,12,22]

Among all randomised and treated subjects we found that the DFUs experienced significantly shorter times to reach closure with active CDO; when stratified by the primary outcome, the CDO arm experienced significantly shorter times to reach 50%, 75% and 100% closure. These results support the findings of others that earlier, aggressive methods of intervention such as CDO are not only beneficial, yet also cost-effective by bringing wounds to closure more quickly.[39,40] In doing so, the increased costs and burden of ongoing care, infection, and potential hospitalisation might be reduced or avoided.[40]

This study had a large number of subjects that were excluded for failing to meet the stringent inclusion/exclusion criteria. These criteria were designed to eliminate most variables that could confound or interfere with investigating the ability of oxygen to aid in the healing of chronic wounds. There were two factors that accounted for the majority of screening failure; the DFUs were too small and because the rate closure during the run-in period was too high once they received good standard of care MWT, debridement and offloading.

It has been shown that DFUs which exhibit a relatively high rate of closure early in a treatment regimen are significantly more likely to close without the intervention of advanced therapies.[41] Sheehan et al. found that wounds experiencing <50% PWAR in four weeks were significantly less likely to close than those experiencing ≥50% PWAR.[40] Similarly, Lavery et al found that wounds experiencing <60% PWAR in four weeks were significantly less likely to close than

those experiencing ≥60% PWAR.[39] In other words, a high PWAR indicates wounds that are easy to close, whereas a lower PWAR is indicative of wounds that are harder to close. Therefore, wounds that exhibit lower run-in rates of closure (indicating that they are more chronic) should be more responsive to the use of advanced modalities such as CDO. As DFUs became increasingly chronic, we found an increasing benefit from CDO. Subjects who experienced lower DFU closure rates during the run-in period experienced significant and beneficial effects, and this benefit was greater as the run-in ulcer closure rates were reduced (a measure of more chronic wounds). The sample set for the lowest run-in ulcer closure rate (<30% PWAR during the two-week period) examined those subjects with DFUs that appear hardest to close. There was a significant and beneficial treatment effect in the CDO arm of 43.9% full ulcer closure versus 13.2% for the placebo arm. These most chronic DFUs were more than three times as likely to close with the treatment of CDO versus standard of care alone. The DFUs in this analysis group experienced relatively low rates of closure, similar to those found to have significantly lower probability of healing in published literature without using advanced interventions such as CDO.[39,40] The results presented here are in alignment with these other studies in that DFUs which exhibit a relatively low rate of closure are significantly less likely to close without intervention of advanced therapies.[41]

Interestingly, CDO appeared to have very similar absolute performance at various levels of wound chronicity, ranging between 46.2% and 43.8% closure rates, whereas MWT experienced a dramatic decrease from 22.6% to 13.2% closure as the DFUs became more chronic, which is what would be expected from the aforementioned literature.[39–41] This suggests that the performance of the CDO arm may be proportional to the chronicity of the wound: more chronic wounds receive more benefit from CDO.

The basis for assessing the effect of wound size is that smaller wounds are more likely to heal within the 12-week study timeframe.[25] In theory, if the CDO arm enhances healing rates, the relative effect between the CDO and placebo arms should be similar or greater in larger wounds than in smaller wounds. The effect of DFU size on primary outcome shows that the CDO arm has an increasing beneficial improvement as the DFU size increases. Both arms show a decrease in the absolute number and percentage of DFUs that close with increased size during the study. This is expected since larger DFUs take longer to heal on average and therefore fewer would heal within a specific timeframe as they become larger. However, as the DFU size increases, the two arms decrease at different rates. The result is that the relative performance of the CDO arm increases with increasing DFU size, indicating that CDO may be of more benefit in larger wounds which are more difficult to heal.

CDO appears to offer a significant benefit to close DFUs in weight bearing areas as compared with placebo. The results for weight-bearing ulcers in the CDO arm reflect that of the overall results for ITT subjects with 33.9% and 46.5% closure (all treated and all completed). However, the results for the placebo arm are much lower (less than half) in the weight-bearing group: 7.5% and 10.0% for ITT weight-bearing versus 16.7% and 22.6% for all ITT subjects. Most of the DFUs that closed in the placebo arm were in non-weight-bearing locations. In weight-bearing wounds, the CDO arm closed more than four times as many ulcers than the placebo arm. Higher local oxygen tensions have been shown to result in proportional increases in the rates of collagen synthesis and tensile strength,[15–17] which could be reasons for the greater effect of CDO in weight-bearing wounds.

With regard to DFU durability, no significant differences were found between the two arms, which may be due to the low number of patients in each arm at this stage. The majority of wounds in the placebo arm that did close were on non-weight-bearing locations (66.7%), whereas the majority of wounds that closed in the CDO arm that closed were on

Table 12. A summary of results compared to three other published studies using advanced therapies to treat diabetic foot ulcers

						Wound closure (%)			
Study	**Test device (therapy)**	**Level of evidence**	**Comparator**	**n**	**Length of study (weeks)**	**Test**	**Control**	**p-value**	**Relative performance**
Niederauer et al	TransCu O2 (CDO)	1A	Placebo device MWT with controlled dressings and offloading	105	12	46.2%	22.6%	0.016	204%
Blume et al 2007[42]	VAC (NPWT)	1B	MWT with alginates, foams, hydrocolloids, or hydrogels	335	16	43%	29%	0.007	148%
Marston et al 2003[43]	Dermagraft (Skin Subst.)	1B	Saline-moistened gauze	245	12	30%	18%	0.03	167%
Edmonds 2009[44]	Apilgraf (Skin Subst.)	1B	Non-adherent dressing	72	12	52%	26%	0.03	200%

The CDO study discussed here (row 1) has level 1A evidence (fully-blinded, prospective randomised controlled trial (RCT) with a placebo). All other studies have level 1B evidence (unblinded, prospective RCT).

weight-bearing locations (83.3%). While not significant, it is interesting to note that the placebo MWT did not close many weight-bearing wounds, even though the original number of weight-bearing wounds was the same between the arms, and a non-weight-bearing wound reopened in this arm. No non-weight-bearing wounds reopened in the CDO arm. Several weight-bearing wounds reopened in the CDO arm. There was no offloading of wounds during the follow-up phase which may account for these wounds reopening. It is encouraging that at the end of the six-month study, including three months of unrestricted activity after completing the treatment phase, twice as many wounds were closed in the CDO arm versus the placebo arm.

The median days for subjects who dropped to reach 50% and 75% wound closure follow similar trends to all treated wounds, with CDO needing fewer days to reach the stated wound closure than placebo at both levels of wound closure. For the days to reach 50% wound closure, the days are lower in the subjects who dropped as compared with all treated subjects; however, the number of subjects was very small, making it difficult to draw any conclusions. For the days to reach 75% wound closure, the days are substantially similar. It is encouraging to see that the subjects who dropped appear to have been following the same trends as compared with the entire treatment population before departing the study.

There were no statistically significant differences in response to CDO based on race or ethnicity, possibly due to the smaller sample sizes. However, as was found for the entire population, the CDO arm was higher than the placebo arm for each racial or ethnic group. The relative performance for each group was similar to the overall relative performance of 209%.

While the adverse events did not show any statistically significant differences between the arms due to the small sample sizes, there were interesting patterns which are in alignment with the findings of others regarding the ability of oxygen to reduce infections in wounds.[20–22] Even though the total number of adverse events were similar, the relative number of adverse events associated with infections of the target study wound were about half in the CDO arm compared with placebo. Furthermore, the severity of the infections in the CDO arm appeared to be less, with the placebo arm requiring four times as many hospitalisations and having two wounds with gangrene, whereas none were experienced in the CDO arm.

Table 12 shows a comparison of our result to other advanced treatments in wound closure, all of which assess wound closure within similar time frames. The absolute performance of 46.2% in the CDO arm compares very favourably to published results from other advanced wound therapies (30%–52%). This comparison becomes even stronger when one considers that the other studies allow for closure by secondary means other than the therapy being studied, including but not limited to surgical closure and amputation. The CDO study is the only study in which the target treatment of interest was used as the sole treatment all the way to full closure. Furthermore, the CDO study is the only study that is fully blinded with a placebo control arm. In all of the other studies, the subjects and physicians were aware of the treatment arm. Only the CDO study eliminates this variable, known commonly as the placebo effect. Given this design, the relative performance of the CDO versus the placebo arms becomes a real measure of benefit. As can be seen in Table 12, the relative performance of CDO (204%) is higher than any other study.

It is important to note that, while the outcomes using CDO are shown to result in significant and beneficial treatment of DFUs, the oxygen flow rate from the CDO device was set to the minimum flow rate of 3ml/hour. Higher flow settings, up to 15ml/hour, could plausibly result in improvements in larger or more ischaemic wounds through the availability of additional oxygen and higher potential oxygen concentrations. Higher local oxygen tensions have been shown to result in proportional increases in the rates of cell proliferation and re-epithelialisation,[13–14] collagen synthesis and tensile strength,[15–17] angiogenesis[18–19] and antibacterial activity through respiratory burst.[20–22] Similar results have been demonstrated in other studies of CDO in various wound types.[13,27–31] Further experience with the device may ultimately reveal that the device benefits patients who need it the most.

Limitations

While this study was the first, to our knowledge, to have a completely double-blind placebo-controlled device as a comparator, it stands to reason that an even larger sample size could allow for different dose ranges of volume of oxygenation as well as different dressing form factors. We look forward to further works that might confirm or refute the results of the present data.

Conclusions

In a fully blinded study, we report a significantly greater percentage of, and rate of, healing in patients receiving CDO therapy compared with a placebo device providing standard wound therapy with identical dressings, debridement and offloading. The results of this study demonstrate that CDO leads to a higher proportion of healed DFUs and a faster time to closure compared with placebo in people with DFUs. Relative performance did not vary significantly with wound size but revealed better relative performance in more chronic wounds and in weight-bearing wounds. Positive correlations are also shown with responses in populations who are more susceptible to diabetes as well as the reduction of severe infections. These results confirm and build upon earlier published results.[37] Multiple outcomes indicate that the more a wound needs CDO therapy, the greater the apparent effect. JWC

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