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

Wael A. Tawfick, MRCSI[1], and Sherif Sultan, MD, FRCS, EBQS-VASC, FACS[1,2]

Abstract

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

Keywords

topical wound oxygen, venous ulcer, compression dressing

Introduction

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

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

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

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

Aim and Objectives

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

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

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

[1] Department of Vascular and Endovascular Surgery, Western Vascular Institute (WVI), University College Hospital, Galway, Ireland
[2] Department of Vascular and Endovascular Surgery, Galway Clinic, Galway, Ireland

Corresponding Author:
Sherif Sultan, Department of Vascular and Endovascular Surgery, Western Vascular Institute, University College Hospital, Galway, Newcastle Road, Galway, Ireland
Email: sherif.sultan@hse.ie

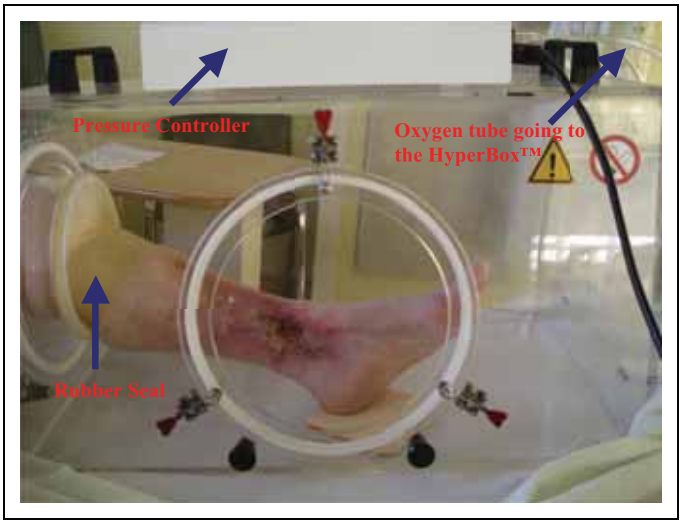

Figure 1. Limb in AOTI-HyperBox. Patient with a medial maleolar ulcer during a TWO_2 treatment session, with the limb placed inside the AOTI-HyperBox. Oxygen and pressure seal is maintained by the rubber cuff, placed below the knee. TWO_2 indicates topical wound oxygen.

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

Inclusion Criteria

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

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

Exclusion Criteria

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

Methods

Study Design

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

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

Techniques

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

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

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

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

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

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

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

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

Table 1. Demographics[a]

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

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

Table 2. Characteristics of the Leg Ulcers[a]

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

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

Table 3. The CEAP Classification[a]

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

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

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

Statistical Analysis

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

Results

Patients

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

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

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

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

Table 4. Previous Ulcer Treatment[a]

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

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

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

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

End points

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

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

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

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

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

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

Of the 67 ulcers, 51 of the TWO_2-treated ulcers showed a reverse gradient of healing, where healing commenced from the core of the ulcer and expanded toward the margin (Figure 6).

Table 5. Results[a]

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

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

This is conflicting to the conventional healing process that initiates from the outward edges of the ulcer inwardly.

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

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

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

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

Discussion

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

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

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

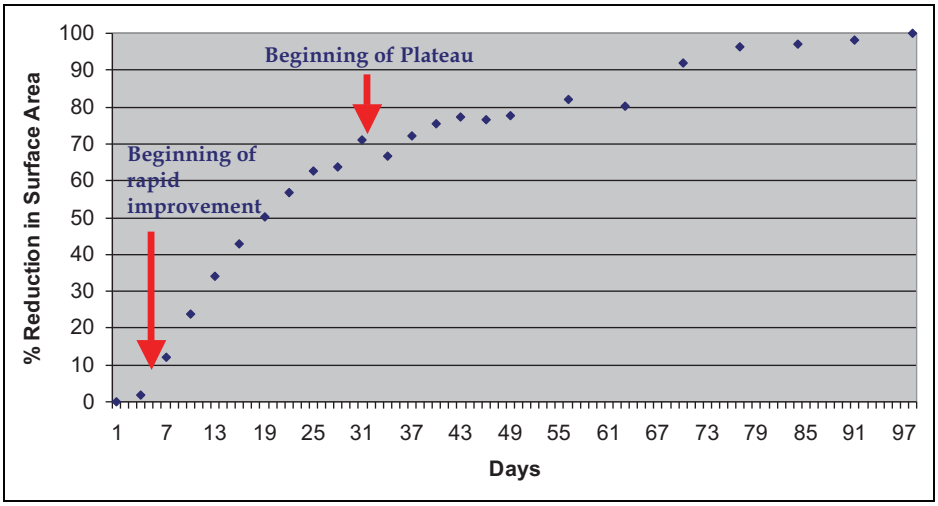

Figure 2. Mean reduction in surface area. There was an initial latent phase up to 5 days, followed by rapid improvement, where the ulcers reached 70% reduction in the surface area. This was followed by a plateau of slow improvement.

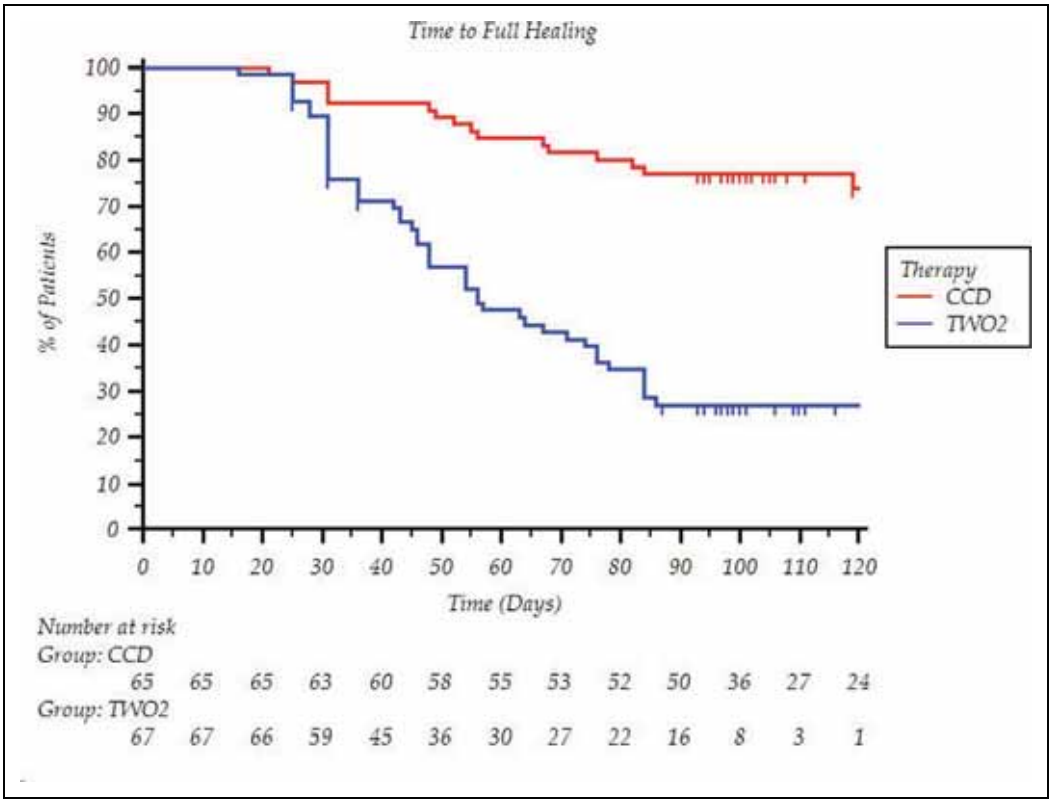

Figure 3. Time to full healing. Kaplan Meier curve showing time to full ulcer healing.
The TWO_2-managed ulcers had a significantly shorter median time to full healing (57days) compared to 107 days in CCD-managed ulcers ($P<.0001$).
TWO_2 indicates topical wound oxygen; CCD, conventional compression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

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

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

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

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

Abbreviations: CCD, conventional compression dressings; TWO_2, topical wound oxygen.

[a] Topical wound oxygen-managed ulcers had a significantly shorter healing time in comparison to conventional compression dressings, regardless of the size of the ulcer or the length of time the patient had the ulcer.

[b] *P* values are analyzed using Mann Whitney *U* test.

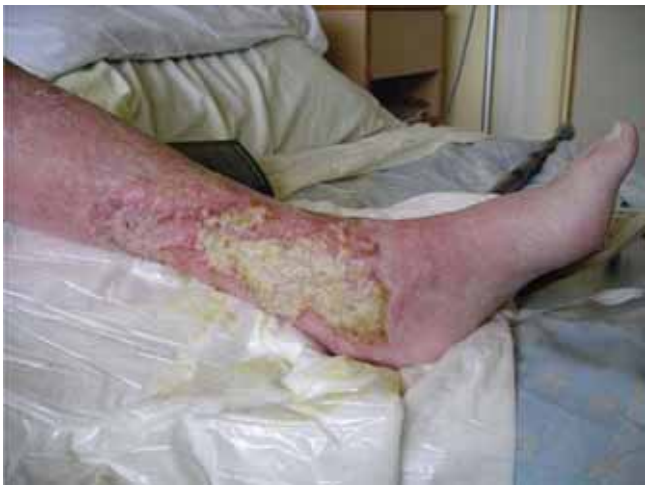

Figure 4. Case 1, Pre-treatment. Large ulcer (98cm^2 surface area) with thick eschar on medial aspect of the leg.

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

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

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

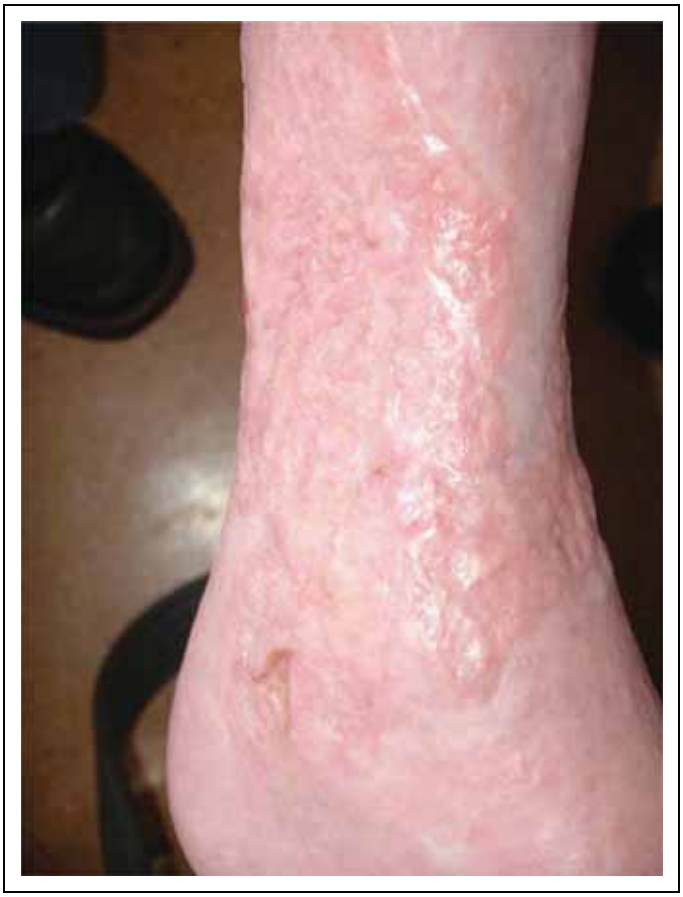

Figure 5. Case 1 after 8 weeks of TWO_2 therapy. Ulcer less than 3cm^2 in the surface area.

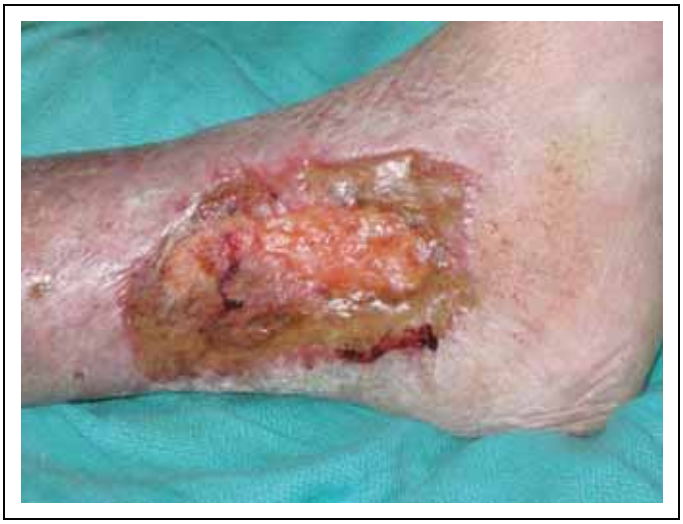

Figure 6. Reverse gradient of healing. Healing starts at the center of the ulcer and then spreads outward.

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

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

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

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

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

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

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

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

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

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

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

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

Conclusion

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

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

Declaration of Conflicting Interests

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

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

Eric Blackman, MD, FRCS(C), FAADEP; Candice Moore, RN; John Hyatt, MD, FRCS(C); Richard Railton, MD, FRCS(C), FACS; and Christian Frye, MD, MPH

Abstract

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

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

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

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

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

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

Dr. Eric Blackman is an orthopedic surgeon; Ms. Moore is a registered nurse and advanced wound specialist; Dr. Hyatt is a vascular surgeon; and Dr. Railton is a general surgeon, St. Catharines Wound Clinic, St. Catharines, Ontario, Canada. Dr. Frye is a consultant for AOTI, Ltd., Galway, Ireland. Please address correspondence to: Dr. Christian Frye, Pittinger Platz 17, 82008 Unterhaching, Germany; email: christian.frye@online.de.

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

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

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

Methods

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

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

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

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

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

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Key Points

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

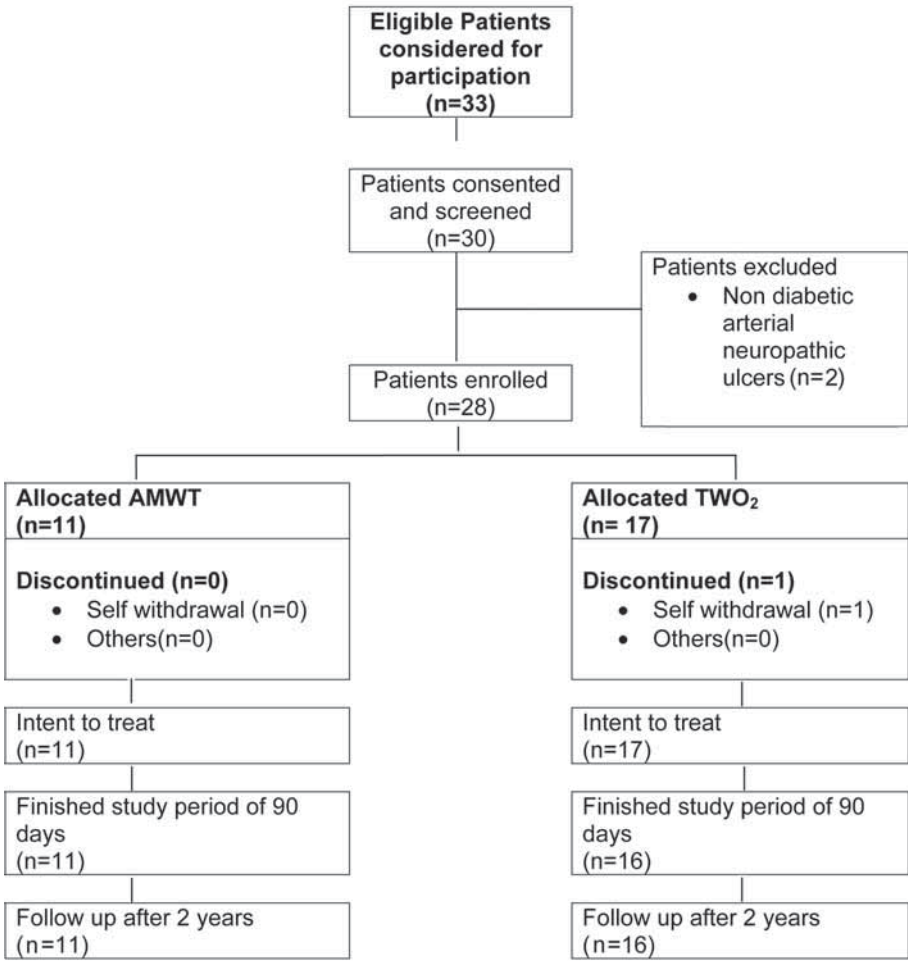

Figure 1. Study population.

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

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

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

Results

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

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

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

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

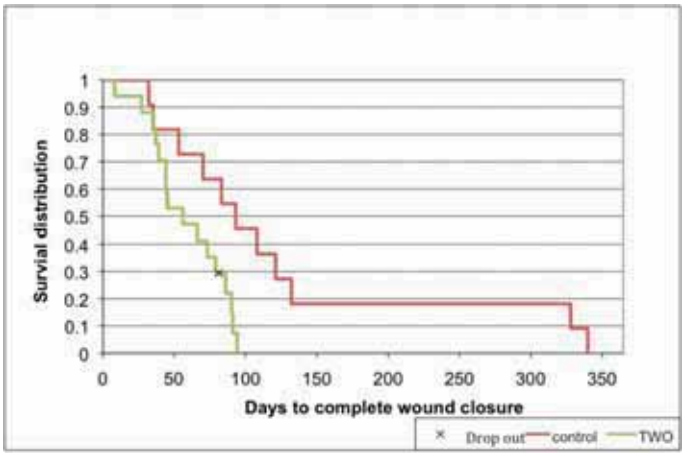

Figure 2. Kaplan-Meier estimate for time to complete wound closure.

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

Discussion

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

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

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

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

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

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

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

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

Table 1. Baseline patient and wound characteristics

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Conclusion

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

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Does Topical Wound Oxygen (TWO_2) Offer an Improved Outcome Over Conventional Compression Dressings (CCD) in the Management of Refractory Venous Ulcers (RVU)? A Parallel Observational Comparative Study

W. Tawfick [a], **S. Sultan** [a,b,*]

[a] *Western Vascular Institute (WVI), Department of Vascular and Endovascular Surgery, University College Hospital Galway (UCHG), Newcastle Road, Galway, Ireland*
[b] *Department of Vascular and Endovascular Surgery, Galway Clinic, Doughishka, Dublin Road, Galway, Ireland*

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KEYWORDS
Topical wound oxygen;
TWO_2;
Compression dressings;
Venous ulcers;
Q-TWiST

Abstract *Objectives:* Topical wound oxygen (TWO_2) may help wound healing in the management of refractory venous ulcers (RVU). The aim of this study was to measure the effect of TWO_2 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 $C_{6,s}$ RVU, assessed by duplex ultrasonography, were managed with either TWO_2 ($n = 46$) 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 TWO_2 managed ulcers were completely healed, compared to 35% of CCD ulcers ($p < 0.0001$). Median time to full healing was 45 days in TWO_2 patients and 182 days in CCD patients ($p < 0.0001$). The pain score threshold in TWO_2 managed patients improved from 8 to 3 by 13 days. After 12-month follow-up, 5 of the 13 healed CCD ulcers

* Correspondence to: S. Sultan, MD, FRCS, EBQS-VASC, Consultant Vascular & Endovascular Surgery, Western Vascular Institute, Department of Vascular and Endovascular Surgery, University College Hospital Galway (UCHG), Newcastle Road, Galway, Ireland. Tel.: +353 91720122; fax: +353 91720121.
E-mail addresses: wael.tawfick@hse.ie (W. Tawfick), sherif.sultan@hse.ie (S. Sultan).

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showed signs of recurrence compared to none of the 37 TWO_2 healed ulcers. TWO_2 patients experienced a significantly improved Q-TWiST.
Conclusion: TWO_2 reduces recurrence rates, alleviates pain and improves the Q-TWiST. We believe it is a valuable tool in the armamentarium of management of RVU.

Introduction

Refractory venous leg ulceration is a common source of morbidity[1,2] and reduced quality of life,[3] especially in the elderly population.[4,5] The prevalence of venous ulcers has been estimated at 0.3% within the UK population,[6,7] with comparable rates in other countries.[5,8–10] There is a probable underestimation of the true extent due to under-reporting.[2]

Venous ulcers are characterized by a cyclical pattern of healing and recurrence,[11] with recurrence rates up to 70% at one year.[12–16]

Venous ulceration places a huge burden on the health-care system.[17] The cost of managing venous ulcers amasses to £400 million sterling per year in the UK.[18] It causes a considerable amount of morbidity amongst patients, with work incapacity, social exclusion and lack of self esteem.[3]

Conventional compression dressings (CCD) are now widely recognised as the main treatment for venous leg ulcers,[19–22] with the addition of surgical correction of superficial venous reflux to reduce recurrence rates.[23,24] 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 (TWO_2) therapy.

The application of positive pressure oxygen to manage open wounds has been studied extensively for decades, demonstrating promising clinical results.[25–33] 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.[34]

The intermittent cycled pressure, under which the TWO_2 is delivered, stimulates circulation, reduces oedema and provides a sealed humidified environment essential for healing.[35] TWO_2 promotes epithelialisation and capillary neoangiogenesis.[34,35] This leads to higher tensile strength collagen being formed during wound healing, which reduces scarring and the risk of recurrence.[36–39]

Objectives

This parallel group observational comparative study was aimed at examining the safety and efficacy of TWO_2 in managing refractory venous ulcers (RVU). We aim to compare the outcome of using TWO_2 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 TWO_2. 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
- $\geq$18 years of age
- Venous ulcer, with normal ankle–brachial index (ABI) $\geq$0.9 and digital pressures $\geq$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[40,41] 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.

Table 1 Demographics. There was no significant difference between both groups in vascular related risk factors, the CEAP class of the patient, or the treatment patients had received prior to the study.

Demographics	TWO_2	CCD	*p* value
Number of ulcers	46	37	
Age (mean/range)	66 yrs (range = 49–83 yrs)	65 yrs (range = 44–87 yrs)	$p = 0.860$
Gender (M:F)	29:17	24:13	$p = 0.524$[a]
Diabetes mellitus	$n = 15$	$n = 11$	$p = 0.484$[a]
Smoking	$n = 4$	$n = 1$	$p = 0.255$[a]
Hypertension	$n = 22$	$n = 15$	$p = 0.330$[a]
MRSA positive	$n = 19$	$n = 17$	$p = 0.251$[a]
Patient referred for primary amputation	$n = 3$	$n = 0$	$p = 0.165$[a]
CEAP class[b]			
$C_{6,s}$	$n = 46$	$n = 37$	
E_p	$n = 33$	$n = 27$	$p = 0.423$[a]
E_s	$n = 13$	$n = 10$	$p = 0.396$[a]
A_s	$n = 10$	$n = 10$	$p = 0.531$[a]
A_p	$n = 7$	$n = 4$	$p = 0.347$[a]
$A_{s,p}$	$n = 29$	$n = 23$	$p = 0.520$[a]
P_r	$n = 33$	$n = 27$	p = 0.423[a]
P_o	$n = 2$	$n = 2$	$p = 0.325$[a]
$P_{r,o}$	$n = 11$	$n = 8$	$p = 0.372$[a]
Previous treatment			
SFJ ligation & division (±perforator avulsion)	$n = 5$	$n = 3$	$p = 0.275$[a]
SFJ ligation, division & LSV stripping (±perforator avulsion)	$n = 19$	$n = 17$	$p = 0.251$[a]
SPJ ligation & division (±perforator avulsion)	$n = 7$	$n = 7$	$p = 0.433$[a]
Multilayer compression dressings	$n = 34$	$n = 21$	$p = 0.214$[a]
Local dressing + Elastic stocking	$n = 8$	$n = 14$	$p = 0.564$[a]
Local dressing + no compression	$n = 4$	$n = 2$	$p = 0.207$[a]

(SFJ = Sapheno-Femoral junction, LSV = Long Saphenous Vein, SPJ = Sapheno-Popliteal junction).
[a] *p* values are Chi-Square.
[b] Basic CEAP classification.[40]

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.

TWO_2 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.

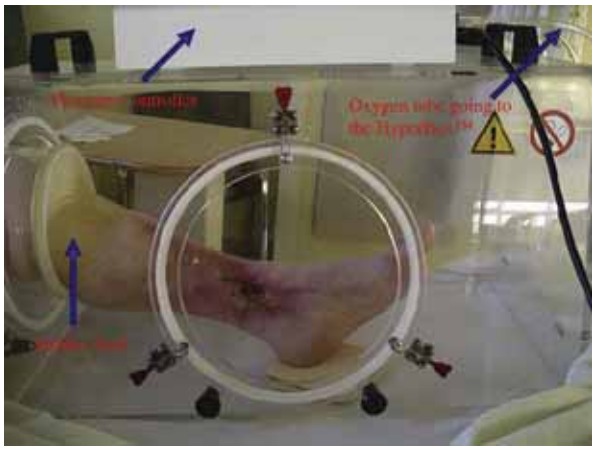

Figure 1 Limb in AOTI Hyper-Box™. Patient with a medial maleolar ulcer during a TWO_2 treatment session, with the limb placed inside the AOTI Hyper-Box™. Oxygen and pressure seal are maintained by the rubber cuff, placed below the knee.

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[44–46]:

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

TOX, TWiST and PROG represented the mean health state duration from Kaplan–Meier analysis; μ_{TOX} and μ_{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.

μ_{TOX} and μ_{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 TWO_2 therapy. 37 limbs with 37 ulcers were managed using CCD. 63% of the TWO_2 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 TWO_2 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 $C_{6,s}$.[40,41]

Using the Venous Clinical Severity Score,[47–49] the mean score in TWO_2 patients was 25, and was 23 in CCD patients.

Following commencement of TWO_2 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 TWO_2 managed ulcers showed a reduction in surface area by 3weeks 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 TWO_2 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 TWO_2 therapy group, compared to 61% in the CCD group.

Table 2 Characteristics 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	TWO_2	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]
Ulcer surface area			
$\leq 5\ cm^2$	$n = 6$	$n = 6$	$p = 0.459$[b]
$6–10\ cm^2$	$n = 7$	$n = 5$	$p = 0.541$[b]
$11–20\ cm^2$	$n = 17$	$n = 12$	$p = 0.423$[b]
$21–40\ cm^2$	$n = 7$	$n = 7$	$p = 0.437$[b]
$\geq 41\ cm^2$	$n = 9$	$n = 7$	$p = 0.584$[b]
Duration of the ulcer			
2–3 years	$n = 10$	$n = 9$	$p = 0.492$[b]
4–5 years	$n = 16$	$n = 10$	$p = 0.303$[b]
6–10 years	$n = 12$	$n = 12$	$p = 0.347$[b]
11–20 years	$n = 6$	$n = 5$	$p = 0.600$[b]
Over 20 years	$n = 2$	$n = 1$	$p = 0.582$[b]

[a] *p* values are Chi-Square.
[b] *p* values are Mann–Whitney *U*.

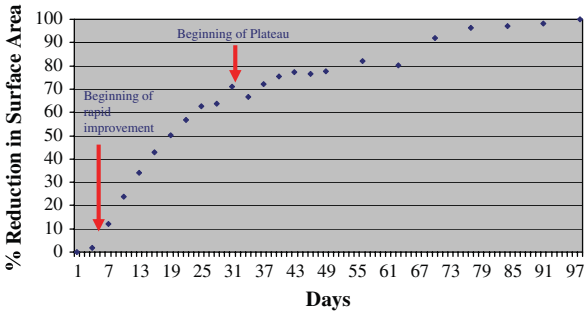

Figure 2 Mean reduction in surface area. There was an initial latent phase up to 5 days, followed by rapid improvement, where ulcers reached 70% reduction in surface area. This was followed by a plateau of slow improvement.

The median time to full ulcer closure was 45 days in the TWO_2 group (95% CI: 39—51), compared to 182 days in the Profore$^{\diamond}$ group (95% CI: 162—203, $p < 0.0001$) (Fig. 3).

Within the TWO_2 group, the duration the patient had the ulcer and the size of the ulcer, did not affect the healing time. TWO_2 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 TWO_2 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 TWO_2 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 TWO_2 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 TWO_2 managed patients improved from 8 to 3 by 13 days.

9 of the 19 MRSA positive ulcers in the TWO_2 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 TWO_2 patients underwent varicose vein surgery, while 5 patients (2 TWO_2 and 3 CCD) underwent redo-varicose vein surgery.

During follow-up, none of the 37 fully healed TWO_2 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.

TWO_2 patients had a significantly shorter mean TOX (1.5 months), in comparison to CCD patients (6 months, $p < 0.001$). TWO_2 patients had a significantly longer mean TWiST (12.5 months), opposed to 4.5 months in CCD patients ($p < 0.001$).

TWO_2 patients had no PROG, in contrast to a mean PROG of 3 months for CCD patients ($p < 0.0001$).

TWO_2 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 TWO_2 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 socio-economic burden of RVU, have motivated the development of alternative therapies as TWO_2 therapy.

The first publication on the use of TWO_2 was by Fischer in 1969.[25] Fischer noted that lesions became aseptic and enhanced granulation was witnessed two days after TWO_2. These findings are similar to our own results. In our study,

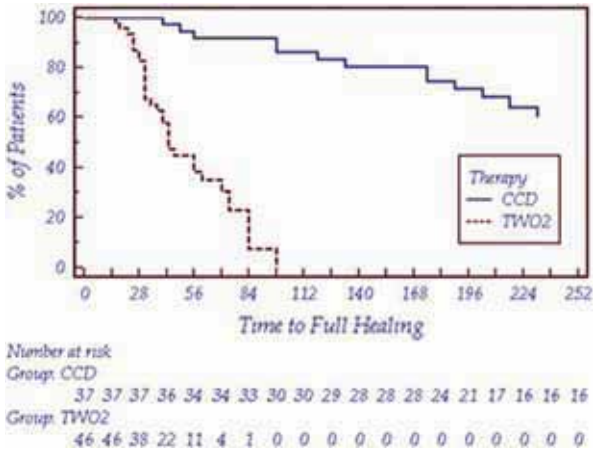

Figure 3 Time to full healing. Kaplan—Meier curve showing time to full ulcer healing. TWO_2 managed ulcers had a significantly shorter median time to full healing (45 days) compared to 182 days in CCD managed ulcers ($p < 0.0001$).

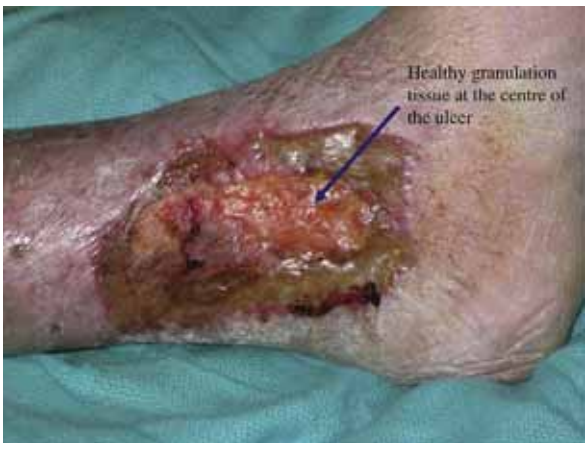

Figure 4 Reverse gradient of healing. Healing starts at the centre of the ulcer & then spreads outwards.

Table 3 Quality Time Spent Without Symptoms of Disease and Toxicity of Treatment (Q-TWiST) was significantly improved in TWO_2 patients.

Time period	TWO_2	CCD	*p* value
TOX	1.5 months	6 months	$p < 0.0001$
TWiST	12.5 months	4.5 months	$p < 0.0001$
PROG	0	3 months	$p < 0.0001$
Q-TWiST	13.625	27	$p < 0.0001$

however, no improvement was witnessed within the first four to five days of TWO_2. 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™ 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 TWO_2 on RVU.[25–33] We believe our study to be the first study on the use of TWO_2 in RVU.[42,43]

In a prospective randomised study by Heng et al, red granulation tissue was present one week after TWO_2.[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 TWO_2.

In both our own study and the Heng study,[32] positive effects could be found, whereas in a study by Leslie et al. no significant effects could be detected.[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[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 TWO_2 was used. These patients were excluded from this study analysis and are not a subset of the 46 patients managed with TWO_2. 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.[42,43]

During TWO_2 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.,[28] showed reduced rates of infection with TWO_2. This depicts our findings, where 9 of the 19 MRSA positive ulcers in the TWO_2 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.[50] In our study 69.5% ($n = 32/46$) of the TWO_2 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.[36–38]

Despite the fact that the mean Venous Clinical Severity Score[47–49] was higher in TWO_2 patients, yet an improved outcome was witnessed compared to CCD patients.

Ulcers that showed no signs of healing in the TWO_2 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.

TWO_2 patients had a significantly improved Q-TWiST compared to CCD patients, denoting an improved outcome ($p < 0.0001$). TWO_2 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.

TWO_2 patients had a significantly longer mean TWiST ($p < 0.0001$). TWO_2 managed patients did not experience any complications from their therapy. There was no recurrence of the ulcers or pain witnessed in the TWO_2 patients.

TWO_2 patients had no time with PROG, compared to a mean period of 3 months of PROG in CCD patients ($p < 0.0001$). In the TWO_2 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

TWO_2 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 TWO_2 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 TWO_2 therapy.

Conflict of Interest/Funding

None.

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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 45days in TWO2 patients and 182days 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 ClosurePlus and ClosureFast Catheters

Natalie Marks, Enrico Ascher, Anil Hingorani, Alexsander Shiferson, 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 (3-7days) documented technical success (complete obliteration, partial obliteration or full patency

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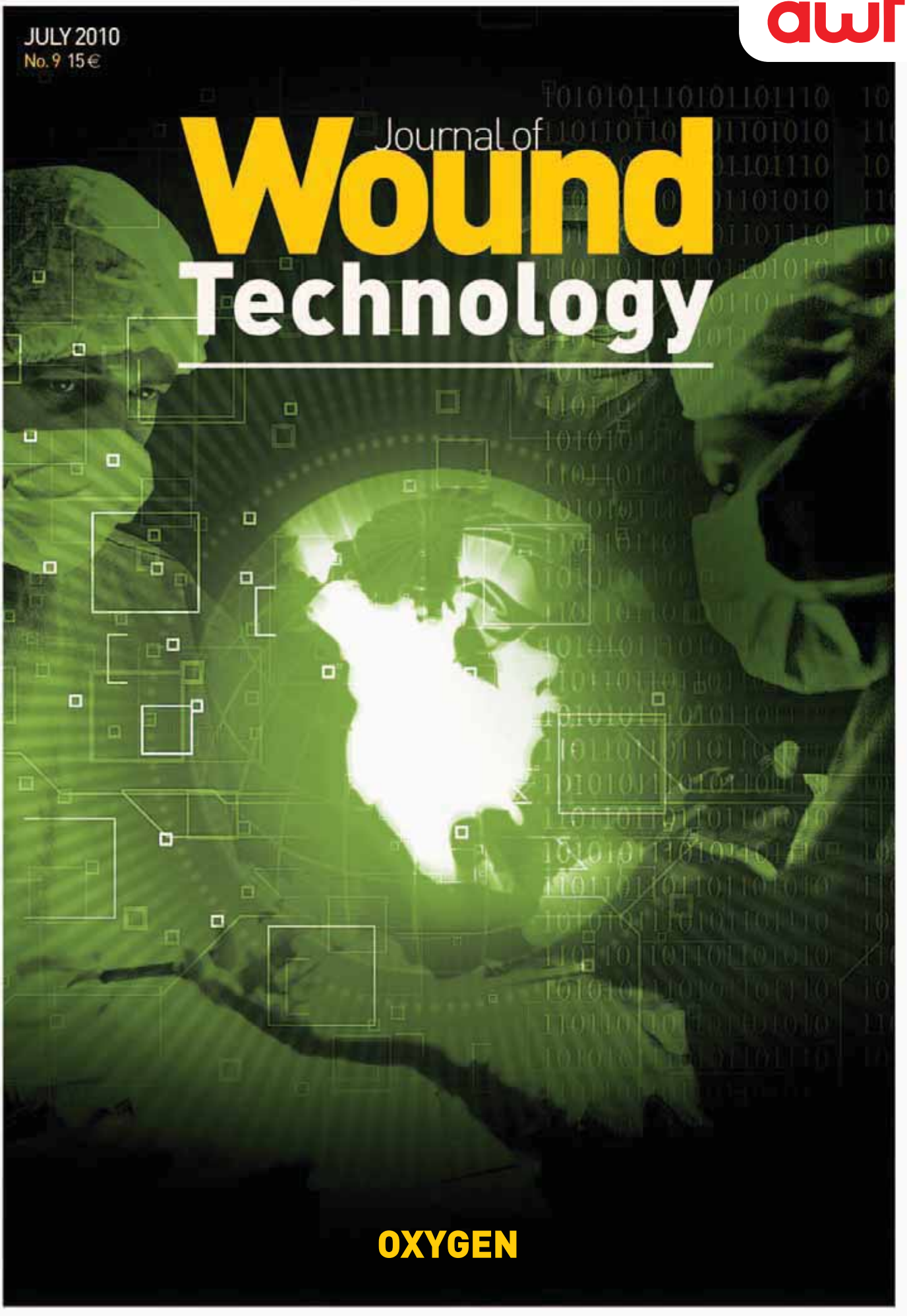

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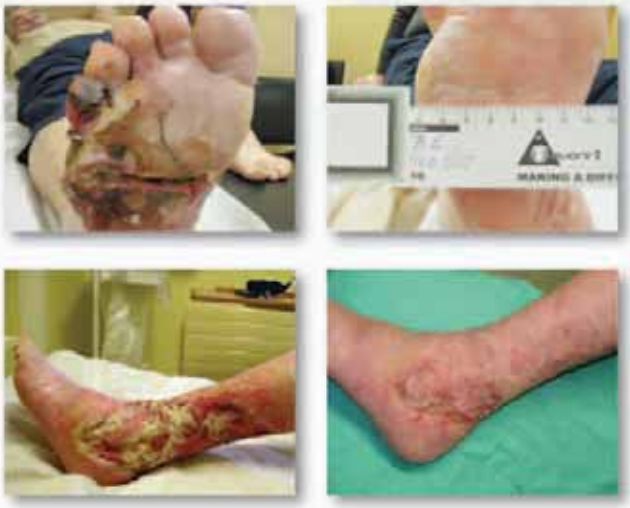

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OXYGEN

"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 (IA) 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.

Finn Gottrup MD, DMSci.
Professor of Surgery, Copenhagen Wound Healing Center

AUTHOR GUIDELINES:
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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*

FINN GOTTRUP MD, DMSCI[1], THOMAS K. HUNT MD[2], HARRIET W. HOPF, MD[3]
1. Professor of Surgery, University of Southern Denmark, Denmark. 2. Professor of Surgery, University of California, USA.
3. Professor of Anaesthesiology, University of Utah, USA.

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.[1,2] 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 (O_2-) and hydrogen peroxide that are injected into phagosomes where they initiate bacterial killing. This, for a while, dominates oxygen consumption, and PO_2 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[3,4] 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 PO_2 of the tissue (P_tO_2).[1,2,5,6] Prolyl hydroxylases have a Km (concentration of substrate resulting in half the maximal rate of enzyme activity) of oxygen of about 25 mm Hg,[7,8] and the production of collagen has been found proportional to P_tO_2.[5,9] 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,[10] 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.[2] 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.[3] 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.[5]

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.[4]

A so-called hypoxia-inducible factor, HIF has been identified as an important trigger for transcription of angiogenetic factors.[10] 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.[4] This is opposite to the usual interpretation of the facts, but it is well defended by Lu and Varma.[4] 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,[11,12] the optimal growth of epidermal cells is found at an oxygen concentration of 10-50%.[1,13,14] Hyperbaric oxygen treatment increases the proliferation of the fibroblasts and the differentiation and epidermopoesis of the keratinocytes, but not the proliferation of keratinocytes.[5]

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_tO_2 tissue of the anal region, where the healing normally is excellent despite massive contamination.

Oxygen at Tissue Level

P_tO_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.[15] P_tO_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 PO_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.[16] 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.[14] 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_tO_2 in a wound after increased F_iO_2 indicates adequate wound tissue perfusion, a useful trick to interpret the meaning of P_tO_2 measurements.[17]

Measurement of P_tO_2 has been performed by introducing a small oxygen sensor in the tissue. Skin and subcutaneous tissue are first tissue to become hypoxic under sympathetic 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.[18]

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.[19] 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 arteriosclerotic 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.[25] 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_tO_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.[26] 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 anastomic leakage after bowel surgery and increased recurrence rate after hernia surgery have been described.[27-29] 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.[30] In such areas the production of collagen is 1.8 times higher in non-smokers compared to smokers (more than 20 cigarettes per day).[31]

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 defects[30,32] 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.[35] 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.[36] Postoperative complications, recovery as well as long term health seem to benefit from smoking cessation lasting at least 4 weeks.[36,37] 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.[37,38] 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.[39] 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.[5,7,9,16] 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 PO_2 and the resulting postoperative wound infection rate has been shown.[17] If a rise of oxygen concentration in the breathing air did not resulted in an increased subcutaneous PO_2, 45% of the patients developed a postoperative infection. If, however, the tissue perfusion was sufficient resulting in an increase of PO_2 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.[17]

In one third of all wound infections the bacteria found are sensitive to the prophylactic antibiotic that had been provided prior to incision.[42] Decreased oxygenation may be the reason for this. Experimental studies have shown that antibiotics and oxygen are additive,[43-45] and antibiotics are lesser effective in hypoxic wounds.[46,47] 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.[48] Using the SENIC score system it was found that 40% of infections occurred in the 55% of patients classified as having uncontaminated wounds.[42] 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.[49] showed that colonic surgery patients benefitted 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.[50] 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.[51] showed that when nitrous oxide is removed and replaced in the breathing mixture by oxygen, wound infections diminished significantly. In contrast, Pryor et al.[52] 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.[53] 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.[54] reported the results of 1400 patients and found no significant difference between infection rates of SSI in patients receiving 80% and 20% inspired oxygen. The authors of these studies did not measure P_tO_2 and cannot, therefore conclude that raising P_tO_2 in wounds has no effect. As with all of the dissenting studies, tissue P_tO_2 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.[55] In the papers showing an effect of supplemental high-inspired oxygen there has been liberal fluid replacement, P_tO_2 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.[56] 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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THOMAS WILD[1], THOMAS EBERLEIN[2], CHRISTIAN FRYE[3]
1. Paracelsus Medizinische Privatuniversität, Salzburg. 2. WoundConsulting, Vienna. 3. AOTI Inc.

Review of Evidence of Topical Oxygen Therapy and Chronic Wounds

Abstract

Chronic wounds represent a significant burden and danger for the affected patient population. Prevalence estimates for pressure ulcers, venous ulcers and diabetic ulcers combined suggest that as many as 3% of the total population are affected by these conditons. Therefore the treatment of chronic wounds is a major challenge for caregivers and places a significant financial burden on the healthcare system. One approach to treatment of these wounds is the Hyperbaric Chamber. The widespread use of this mehod is however limited by costs and a number of therapy associated risks. In order to eliminate some of the problems associated with systemic hyperbaric oxygen therapy, a novel approach using topical oxygen has been launched and is discussed in this paper. Chronic wounds are mostly associated with an absolute and relative lack of oxygen. Oxygen plays a key role in the antibacterial response mechanisms as well as a substrate for important repair mechanisms. Latest research indicates that free oxygen (02) radicals are important for cell signaling (redox signaling). The physiological reasoning of the importance of 02 seems to be reflected in clinical studies. The results of about 1,250 patients were published in 27 identified studies of varying quality. The latest studies eliminated the majority of critical issues of the older papers. In addition they seem to underline the advantages of applying oxygen through a cyclical pressure. In conclusion the approach is very promising but there is a clear need for well designed randomized clinical trials.

Keywords: chronic wounds, topical oxygen, literature review, diabetic wounds, hyperbaric oxygen, oxygen and woundhealing

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 (TWO_2).[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 TWO_2 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 (O_2) is one of the major prerequisites for life. In mammals, all processes at the cellular level require O_2 which is provided in the majority via the adenosine triphosphate (ATP) pump. ATP cannot be stored and its synthesis requires 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 O_2 via the circulatory and respiratory systems the largest human organ however is partly supplied with 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 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

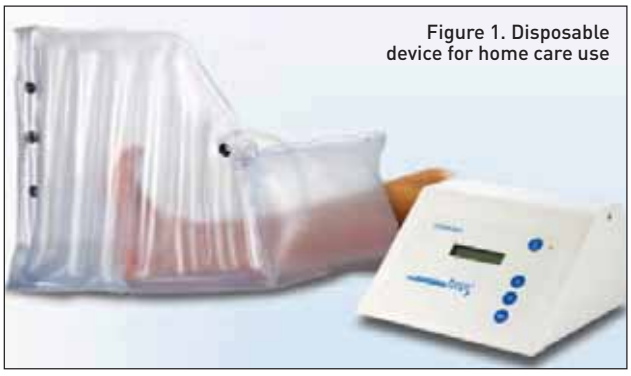

Figure 1. Disposable device for home care use

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 O_2 to fulfill their purpose. But O_2 is not only the main source of energy.

In all phases of wound healing O_2 is also needed as a substrate for essential enzymatic process. 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 oxidase can increase the O_2 consumption by as much as 50-fold. Up to 98% of the oxygen consumption of neutrophils is needed for ROS production[30]. Newer research indicates that free O_2 radicals are important for cell signaling to stimulate cell migration, cell proliferation and neovascularisation.[31]

A means to describe the amount of O_2 available is its partial pressure (pO_2). While the normal pO_2 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 pO_2's at the wound center. Trauma can destroy capillaries altering the diffusion distance for O_2. 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 O_2 within the chronic 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 O_2 necessary to achieve half maximal ROS production (the Km) is in the range of 45–80 mmHg, with maximal ROS production at pO_2 at > 300 mmHg.[30] As the pO_2 in the center of the wound is regularly below a pO_2 of 10 mmHg, the maximal effects of respiratory burst-dependent wound infection management can only be achieved through the administration of supplemental O_2 to attain wound pO_2 levels beyond those encountered when breathing room air.[35] This also explains why the state of wound tissue oxygenation is a sensitive indicator for the risk of infection in surgical patients.[36,37]

Figure 2. Rigid device for hospital and institutional use

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 O_2 as a substrate in different enzymatic processes. Three peptide chains are hydroxilated 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 O_2 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 pO_2 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 pO_2 is as high as 66.[38] As the pO_2 in the center of the wound is regulary below a pO_2 of 10 mmHg, hypoxic wounds deposit collagen poorly and are more likely to become infected.[38]

Systemic hyperbaric therapy with pressures up to 2.5 atmospheres (2.500mbar) enhances the arterial pO_2 multiple but requires an intact capillary network to enhance the wound pO_2. Consequently, local tissue oxygenation seems reasonable as no intact vasculature is needed. Unfortunately O_2 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 pO_2 in the tissue.

Modern topical oxygen devices (like AOTI – Advanced Oxygen Therapy Inc.) address this problem with 2 components. First, highly concentrated O_2 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 O_2. In 1975 Fisher measured the capillary pO_2 in the finger tip as a comparison.[6] The pO_2 in the capillaries of the wound was less than 80mmHg at start and using a topical oxygen device with a pressure of 22mmHg the pO_2 in the wound capillary was raised after one hour to 115mmHg and 120mmHg after two hours. The fingertip pO_2 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.[7] He measured the pO_2 in granulation tissue near the wound surface and at a depth of 1 mm. pO_2 in the plasma of the wound surface was raised from 50mmHg to 450mmHg and fell down to 50mmHg 2 minutes after stopping the O_2 therapy. Olejniczak reported about great difficulties to measure the pO_2 at 1mm depth back in 1976. He didn't observe a raise of the pO_2 during the therapy using 12mmHg pressure in the delivery device. When using nitrogen as a gas for the topical application the pO_2 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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NR.	AUTHOR/YEAR	TITLE	STUDY DESIGN	WOUND ETIOLOGY	RESULTS
1	Gorecky, 1964	Oxygen Under Pressure Appllied directly to Bed Sores: Case Report	Case study with on patient	Pressure ulcer	2 Ulcer with no tendency to heal for 9 moth healed within 4 month under TWO_2
2	Fischer, 1966	Low Pressure Hyperbaric Oxygen Treatment of Decubit and Skin Ulcers	Cas study	15 patients with meningocele, diabetic/ arteriosclerotic ulcers and pressure	Very good healing in all cases
3	Fischer, 1969	Topical hyperbaric oxygen treatment of pressure sores and skin ulcers	Case study with 58 patients and controlled with 6 patients	Diabetic ulcers (2), venous ulcers (19), pressure ulcers (29), ischaemic (6), trauma (2)	52/58 heald completely. 4 out of 6 wound that did not heal had underlying osteomyelites unknown at therapy begin
4	Torelli, 1973	Topical Hyperbaric Oxygen for Decubitus Ulcers	Description of treatment and case study	70 pressure ulcers	Practical and safe method with very good results on pressure ulcers
5	Fischer, 1975	Treatment of ulcers on the legs with hyperbaric oxygen	Case study with 30 patients	All wound on the lower extremeties. (5), pressure ulcer (16), venous ulcer (3), post surgical (2), rheumatoide arthritis (3), hyper-gamma-globulinaemia (1)	28/30 wounds healed completely
6	Olejniezak, 1976	Topical Oxygen Promotes Healing of Leg Ulces	Case study with 174 of various etiologies	Venous ulcers (102), arteriosclerotic ulcers (33), post surgical (33), sickle cell anaemia (4), lupus erythematodes (2)	Improvement in all wounds. 96% healing in venous wounds, 70% in ischemic ulcers
7	Diamond, 1982	The effect of Topical hyperbaric oxygen on lower extrimemity ulcerations	Case study	11 patients with wounds of various etiologies	Healing in "all cases"
8	Heng, 1983	Hyperbaric oxygen therapy for a foot ulcer in a patient with polyarteritis nodosa	Case study	1 patient with ulcer and panarteritis nodosa	Healing
9	Heng, 1984	Hyperbaric oxygen therapy for pyoderma gangrenosum	Case study	2 patients with multiple ulcers on lower extremities and pyoderma gangaenosum	Healing in both cases after 6 and 12 weeks
10	Heng, 1984	A simplified hyperbaric oxygen technique for leg ulcers	Prospective, controlled study	Iscaemic wounds	5/6 patients in the TWO_2-group with 27 wounds healed 3 weeks vs. 0/5 in the control group
11	Ignacio, 1985	Topical oxygen therapy treatment of extensive leg and foot ulcers	Case study	15 patients of which 12 had diabetis ulcers, 12 osteomyelitis, 1 elephantiasis and 2 charcot feet	11/15 patients healed (73%)
12	Lehmann, 1985	Human Bite Infections of the Hand: Adjunct Treatment with Hyperbaric Oxygen	Semi-Randomized controlled study	43 patiens with human bite wounds. 16 patients TWO_2 and 27 served as controls	Hospital stay was shortened from 4,7 days vs. 11,2 days in the control group
13	Upson, 1986	Topical hyperbaric oxygenation in the treatment of recalcitrant open wounds. A clinical report	Case study	2 patients with arterial ulcers	Both healed
14	Leslie, 1988	Randomized controlled trial of topical hyperbaric oxygen for treatment of diabetic foot ulcers	Prospective randomized study over 2 weeks	28 patiens; 12 in TWO_2 group 16 controls	More than 55% reduction in both groups. No significant difference

NR.	AUTHOR/YEAR	TITLE	STUDY DESIGN	WOUND ETIOLOGY	RESULTS
15	Landau, 1988	Topical Hyperbaric Oxygen and Low Energy Laser Therapy for the treatment of diabetic foot ulcers	Case study	50 patients with diabetic ulcers. 15 patients were only treated with TWO_2 and 35 35 in combination of TWO_2 and low energy laser	43/50 patients healed
16	Heng, 2000	Angiogenesis in necrotic ulcers treated with hyperbaric oxygen	Prospective, randomised study	40 patients with mainly pressure ulcers. Many of which associated with diabets and osteomyelitis	90% healed in the TWO_2 group vs. 22% in the controls
17	Heng, 2000	Enhanced healing and cost-effectiveness of low-pressure oxygen therapy in healing necrotic wounds: A feasibility study of technology transfer.	Case study / virtual control group	15 patients with 24 wounds of different orign, 4 patients with osteomyelitis	22 out of 24 ulcers healed within 12 weeks. Significant cost reduction in the TWO_2 treated patients
18	Landau, 2001	Topical Hyperbaric Oxygen and Low Energy Laser Therapy for Chronic Diabetic Foot Ulcers Resistant to Conventional Treatment	Case study	100 patients with diabetic ulcers treated with TWO and low energy lase	81% healed
19	Edsberg, 2002	Topical hyperbaric oxygen and electrical stimulation: exploring potential synergy	Case study	8 patients with pressure ulcers grade III and IV.	6/8 wounds healed within 16 weeks
20	Edsberg, 2002	Reducing epibole using topical hyperbaric oxygen and electrical stimulation	Fallstudie	1 patient with grade IV pressure ulcer	Healed
21	Kallianen, 2003	Topical oxygen as an adjunct to wound healing: a clinical case series	Case study	58 wounds of various aetiology on 32 patients	65% healed without surgical intervention. 72,2% with surgical intervention (surgery/flap/graft)
22	Ishii, 2004	Efficacy of topical hyperbaric oxygen for refractory foot ulcer	Case study	2 patiens with unspecified	Both wounds healt 3 and 9 month
23	Landau, 2006	Topical hyperbaric oxygen and low-energy laser for the treatment of chronic ulcers	Case study	274 patients; 218 patients with diabetic ulcer and 156 with venous ulcer	78% healing in both groups
24	Gordillo, 2008	Topical oxygen therapy induces vascular endothelial growth factor expression and improves closure of clinically presented chronic wounds.	Controlled study	57 patients; 32 HBO vs. 25 TWO_2. Wounds of different etiologies	HBO didn not reduce wound size. TWO_2 reduced wound size and lead to higher VEGF
25	Tawfick, 2009	Does Topical Wound Oxygen (TWO_2) Offer an Improved Outcome Over Conventional Compression Dressings (CCD) in the Management of Refractory Venous Ulcers (RVU)?	Controlled study	83 patients with venous ulcers. 46 patients with TWO_2 and 37 controls receiving compression dressings	80% of TWO_2 treated patients healed vs. 35% in the controls within 12 weeks
26	Aburto, 2010	A Randomized Controlled Trial to Evaluate Different Treatment Regimes with Topical Wound Oxygen (TWO_2) on Chronic Wounds	Randomised, controlled study	20 diabetic ulcers and 20 venous ulcers. Every patient received TWO_2 for 4 weeks. After randomization each 10 patients continued with TWO_2 vs. advanced wound care in controls	Diabetic ulcers: 90% vs. 50% healing; Venous ulcers: 50% vs. 30% healed in 12 weeks
27	Blackman, 2010	Topical Wound Oxygen Therapy in the Treatment of Severe Diabetic Foot Ulcers: A Prospective Controlled Study	Controlled study	28 patients with diabetic ulcers 17 received TWO_2 and 11 advanced dressings	82% of TWO_2 patients healed within 90 days vs. 43% in the controls

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→ how important the atmospheric O_2 is for the supply of O_2 for the skin. Almost 30 years later Fries[33] measured the diffusion of O_2 using a device utilizing a higher pressure than Ollejniczak. He measured the pO_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_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.[25,34]

Evidence of TWO_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.[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_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,[40] 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_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_2) to levels where various enzymes can effectively start healing. The effectiveness of Topical Wound Oxygen (TWO_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_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. ■

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TOPICAL WOUND OXYGEN THERAPY (TWO2) AOTI Inc.

☑ FDA CLEARANCE ☑ CE MARKING

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.

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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 (TWO2) Single-use Sacral Unit AOTI Inc.

☑ FDA CLEARANCE ☑ CE MARKING

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.

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.

PRESENTATION / DIMENSIONS

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

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INDICATIONS

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- Diabetic ulcers
- Venous ulcers
- Skin grafts
- Burns
- Frostbite

CONTRAINDICATIONS

N/A

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

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

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

ABSTRACT

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

Key words: Evidence-based • Oxygen therapy • Pressurised

INTRODUCTION

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

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

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

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

Key Points

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

Authors: HL Orsted, RN, BN, ET, MSc of Wound Healing and Tissue Repair - Principal eQuadra Solutions Inc; R Poulson, Board Certified Respiratory Therapist, Respiratory Care Practitioner, Hyperbaric Technologist, Advanced Negative Pressure Wound Therapy, Associate of the American Professional Wound Care Association; the Advisory Group

Address for correspondence: HL Orsted, Calgary, Alberta, Canada

Email: hlorsted@gmail.com

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

Key Points

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

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

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

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

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

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

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

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

WHAT IS TOPICAL PRESSURISED OXYGEN THERAPY?

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

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

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

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

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

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

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

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

USING NEW TECHNOLOGIES

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

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

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

Key Points

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

Key Points

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

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

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

METHODOLOGY – THE DELPHI METHOD

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

The advisory group

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

The interprofessional members of the AG are

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

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

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

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

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

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

Delphi method

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

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

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

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

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

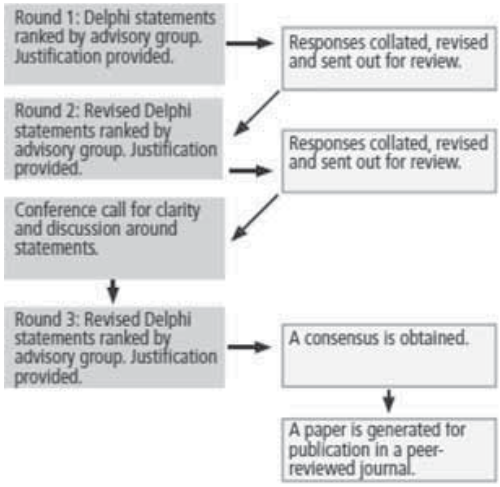

Figure 1. The Delphi method.

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

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

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

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

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

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

THE RESOURCE MATERIAL

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

Table 1 Advisory group resource material

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

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

STANDARD STATEMENTS

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

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

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

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

DISCUSSION OF THE EVIDENCE

Product description

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

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

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

Table 2 Interpretation of the evidence

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

Table 3 Topical pressurised oxygen therapy: quick reference guide

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

DVT, deep venous thrombosis.

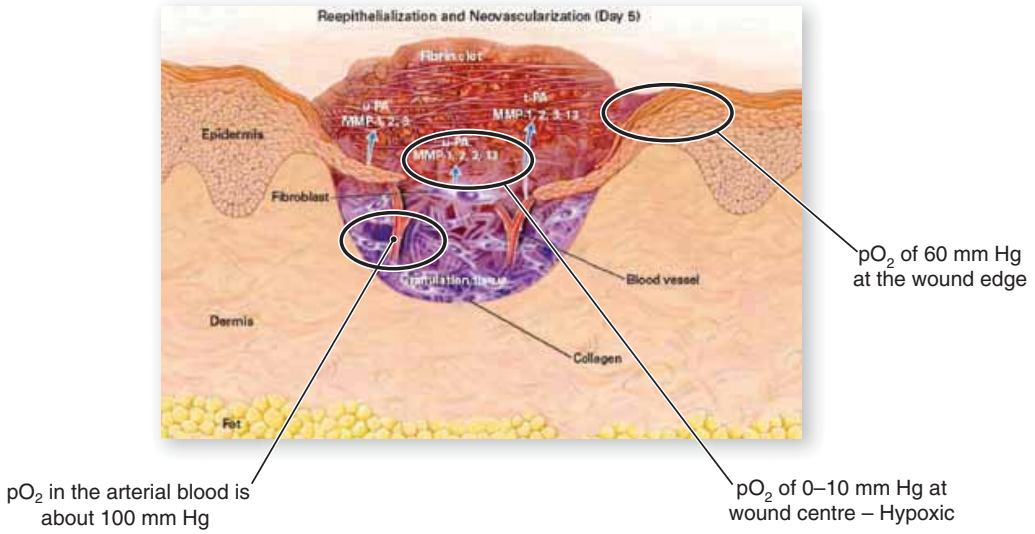

Figure 2. Oxygenation and trauma. Reprinted with permission from reference 10.

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

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

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

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

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

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

Patient selection

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

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

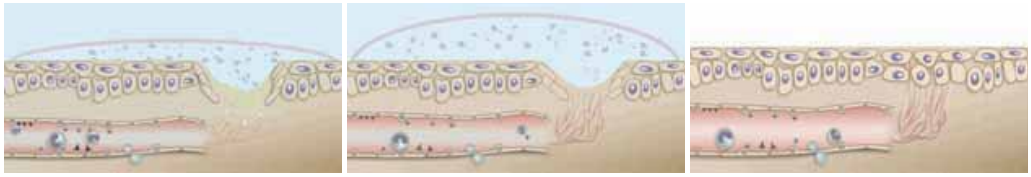

Figure 3. Oxygen penetrates to the cellular level supporting angiogenesis and enhancing collagen formation. Reprinted with permission from reference 10.

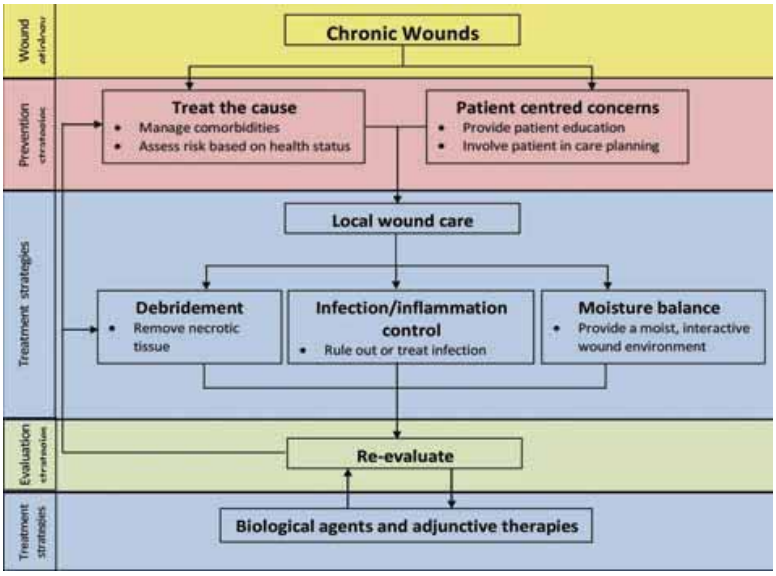

Figure 4. Wound bed preparation paradigm. Adapted with permission from reference 14.

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

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

Patient preparation

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

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

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

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

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

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

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

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

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

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

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

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

Table 4 Recommended protocols for topical pressurised oxygen therapy.

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

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

Application principles

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

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

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

Evaluating treatment

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

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

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

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

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

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

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

Expected outcomes

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

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

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

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

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

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

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

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

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

Resources

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

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

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

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

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

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

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

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

Safety and precautions

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

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

FURTHER STUDIES

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

Key Points

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

Key Points

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

oedema and decreased bacterial burden also require further research.

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

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

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

CONCLUSION

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

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Topical wound oxygen therapy for chronic diabetic lower limb ulcers and sacral pressure ulcers in Japan


Authors:
Hitomi Sano, Shigeru Ichioka

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.

Hitomi Sano *is Plastic Surgeon, University of Tokyo, Japan and Visiting Researcher, Saitama Medical University Hospital, Japan;*
Shigeru Ichioka *is Professor of Plastic Surgery, Saitama Medical University Hospital*

The global incidence of chronic wounds has increased in recent years, driven by the increase in aging and bed-bound populations, with a concomitant upsurge in peripheral arterial disease and diabetes. The lack of adequate tissue oxygenation stemming from poor blood circulation is a common characteristic of diabetic and pressure ulcers. Oxygen plays an important role in the wound healing process[1,2], as well as in infection control[3-5]. Enhanced wound healing and a reduced bacterial burden are thus expected to be advantageous outcomes following the direct local administration of oxygen to chronic ulcers of the skin[6-10].

Local topical oxygen wound therapy is widely employed in North American and European countries, with good reported efficacy [7,11,12]. In late 2010 after Institutional Review Board (IRB) approval, our research group at the Saitama Medical University Hospital (Saitama, Japan) initiated the first clinical investigation of this therapy in Japan and introduced this treatment with two case reports[13,14]. These reports looked at the efficacy of topical oxygen wound therapy in inducing healthy granulation tissue, improvement of transcutaneous oxygen tension and successful wound closure in patients with diabetic foot wounds and pressure ulcers. The following case report follows up on the initial study with results from six patients who presented at the hospital with either chronic diabetic leg or foot ulcers, or sacral pressure ulcers, providing full details of one case.

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.

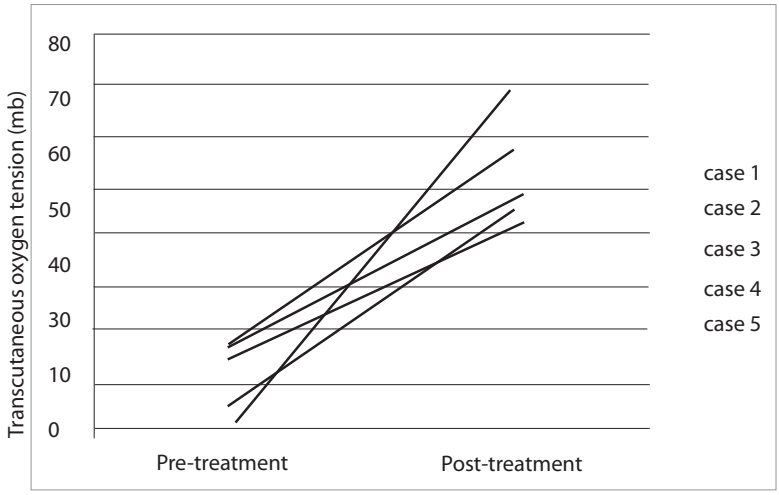

Figure 2. Oxygen tension before and after topical wound oxygen therapy. Pre- and post-treatment TcPO2 values surrounding the ulcers showed an increase in oxygen tension at 1 day after the initiation of therapy.

Table 1. Patient data

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

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

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 reccurence 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

5) showed no clinical improvement following topical wound oxygen therapy *[Table 2]*. In Case 4, the outcome was unknown because the patient voluntarily discharged himself after receiving debridement and 2 weeks of topical wound oxygen therapy, before completing the recommended 4-week treatment protocol. In Case 5, amputation of the affected lower limb was ultimately required as a result of severe local infection that could not be controlled.

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 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

Table 2. Patient outcomes

Case No.	Ischaemia	Revascularisation	Clinical outcomes
1	No	No	Healed by skin graft, no re-currence during 12 months
2	Yes	No	Healed by skin graft, no recurrence during 4 months
3	Yes	No	Healed spontaneously, no recurrence during 2 months
4	Yes	Yes	Self-discharged
5	Yes	Yes	Lower limb amputation
6	No	No	Healed by skin graft, no recurrence during 8 months

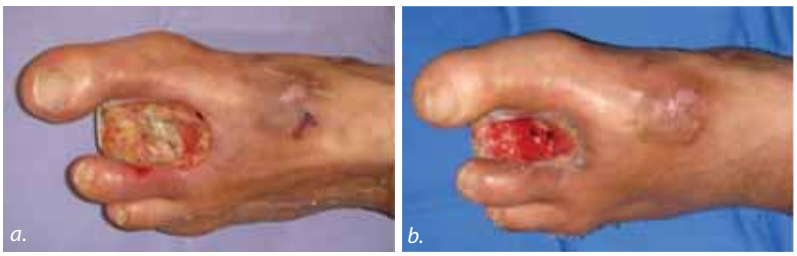

Figure 3. Diabetic foot ulcer; a. The debrided wound is shown prior to commencing topical wound oxygen therapy. b. The wound showed formation of healthy granulation tissue at 4 weeks after the commencement of oxygen therapy.

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 reccurrence rates after 18 months, which was likely related to the augmented patency of the interlaced collagen fibers produced in the high-oxygen environment[7].

Tawfick and Sultan[8] 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[7] 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[19].

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.

WINT

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.*

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Expert commentary: role of oxygen role in wound healing

American 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[1] 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[2]. First results were published in 1966 and three years later. *The Lancet* printed a report on 56 patients treated successfully with topical wound oxygen[3].

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 und 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[4]. 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[5,6].

Oxygen delivery is a critical element in the healing of wounds. The pathophysiology of lack of oxygen in wounds is proven 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

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Author: *Tom Wild, General Surgeon; Wound Center Dessau-Rosslau, Department of Dermatology, Dessau Municipal Hospital Dessau, Germany*

A Randomized Controlled Trial to Evaluate Different Treatment Regimes with Topical Wound Oxygen (TWO_2) on Chronic Wounds

Aburto I[1], Frye C[2]
[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 (TWO_2) device in other studies(2,3) we investigated whether 4 weeks of TWO_2 treatment and consecutive 8 weeks of advanced moist wound treatment (AMWT) is equally effective in healing chronic wounds as continuous treatment with TWO_2.

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 TWO_2 for a period of one month. Then the groups were randomized to continue with TWO_2 (TWO_2-TWO_2 group) or receive AMWT for 2 more month (TWO_2-AMWT group). TWO_2 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 TWO_2-TWO_2 group healed within 90 days vs. 40% in the TWO_2-AMWT group. Patients with CVU had 50% healing vs. 30%, respectively.

Conclusion

Patients with complicated ulcers benefit from the treatment of topical localized oxygen (TWO_2). Continuous TWO_2 treatments for 12 weeks showed significant better outcomes than a shorter TWO_2 treatment regime of 4 weeks followed by AMWT.

Figure 1: Number of closed wounds in different ulcers in TWO_2 group and control group

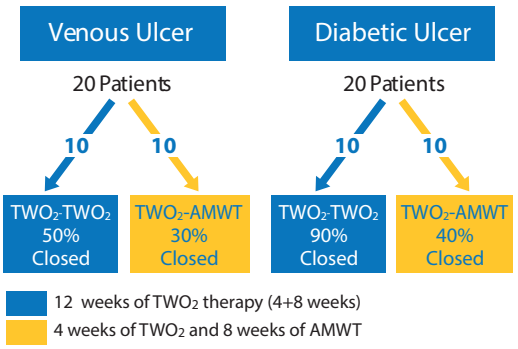

Figure 2: Reusable TWO_2 extremity chamber

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Presented at EWMA 2010 May 26-28, Geneva Switzerland

Topical Oxygen Treatment (TWO_2) 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 TWO_2 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 contious pressure of 30mbar 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. TWO_2 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 TWO_2™ therapy was administered further to support the standard local treatment. The wound showed good granulation tissue after a few days. TWO_2 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 TWO_2 the wound healed drastically quicker compared to the previously used treatment. Within 3 weeks the wound was closed.

Case 1: May 27

Case 1: June 21

Case 2: April 26

Case 2: May 4

Conclusion

TWO_2 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.

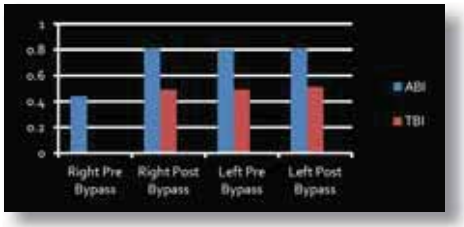
Presented at EWMA 2010 May 26-28, Geneva Switzerland

The Use of Topical Wound Oxygen (TWO2) in a Complicated Acute Venous Embolism and Thrombosis of the Lower Extremity

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

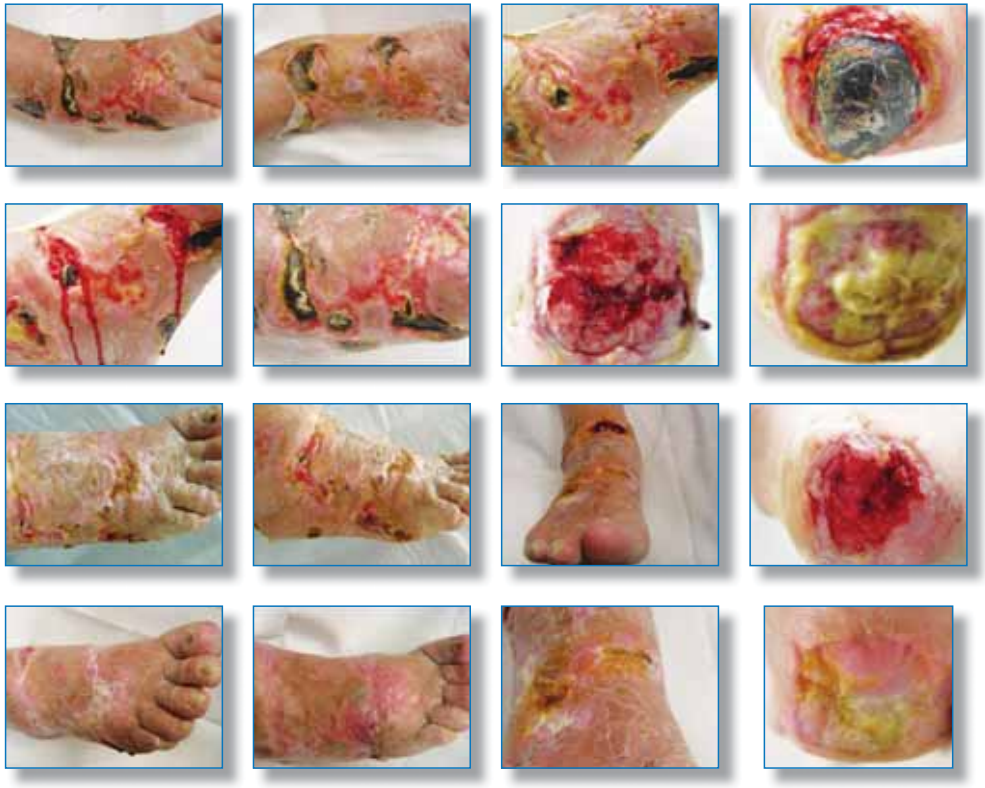
A 66 yr/o Male underwent a Femoral-Popliteal Bypass for a non healing right dorsal foot wound. Eight days following the procedure, the patient developed right lower extremity thrombosis resulting in the formation of deep sub dermal eschars. The patient was admitted for leuckocytosis and wound management.

PMH: PVD, HTN, Obstructive Chronic Bronchitis
Smoking: 1 PPD / 40 pack year hx



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

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



TIMELINE

S/P 3 weeks 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

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

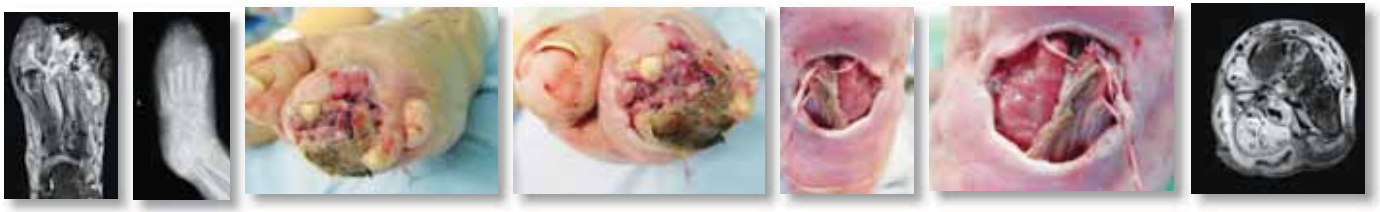
The Use of Topical Wound Oxygen (TWO2) in a Complicated Post Surgical Transmetatarsal Amputation with Incision and Drainage of the Foot

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.



Vitals
Tm:98.5
R:20
HR:104
BP:125/79

Culture and Susceptibility
*Streptococcus angiosus
Blood Culture
• + growth Coag negative staph x 2 initially
• negative growth x 3 following
• Susceptible to Vancomycin and Cefazolin

Labs
WBC:13.3
H/H:7.9/25.3
Plt:729
Na:123
K:4.7
BUN:11
Cr:0.68
Glu:892
HbA1c:14.3
PTT:27.5
INR:1.0

S/P 1 Week

Wet to Dry Saline Packing BID: TWO2 started immediately Negative pressure for 3 days only

S/P 3 Weeks

S/P 5 Weeks

Hgb	7.9	8.8	8.2	8	9.8	8.2	8.4
Hct	25.3	17.2	25.0	25	29	25	25.1
WBC	13.3	17.9	16.3	12.3	11.1	10.8	10.2
	Admission	Post op Day 1	Day 2	Day 3	Day 4	Day 5	

S/P 8 Weeks

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 STVHCS: 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.

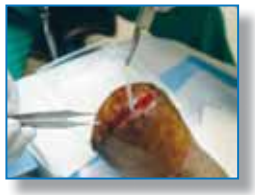
The patient underwent a Vascular Bypass Graft 5 months prior to the TMA procedure. Pre and Post NIVs were N/C. The patient was not a candidate for further vascular surgery and presented with a natural hx of limb loss on the contralateral side prior secondary to PVD and infection.

ABIs: Left Not compressible (N/C)
TBIs: (TMA)
S/P: SFA-PTA Bypass
Non palpable pulses
Doppler: 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.

Time of Healing	X-rays	Blood Cultures	Lab Analysis
9 Weeks	negative	negative	WBC / CPR / Sed rate WNL

S/P 5 Weeks TWO2 Therapy 45 min/BID

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, MD VAMC, 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 (1month-6 month) and average number of treatments to closure at 45 (10-105).

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

Patient 1- DEHISED SURGICAL WOUND

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

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

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

Patient 3- DEHISED SURGICAL WOUND

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

Patient 4- MEDICAL PATIENT WITH PROSTATE CANCER

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

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

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

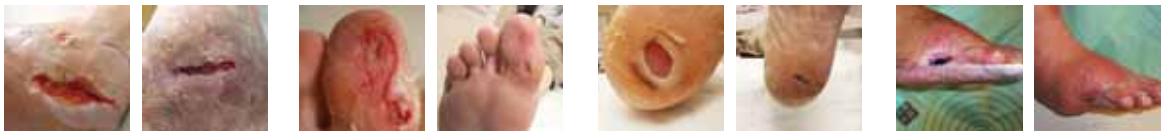
Patient Data

Patient	Topical O2 Treatment (months)	Ulcer Duration (months)	Initial (cm²)	Ulcer Area (cm²)	BMI	Side (R/L)	Gender (M/F)	Age (Years)
		x=	x=	x=				
5	1	18	5.4	1.6	0.8	R	M	59
X	4	3	6.8	3.6	0.8	L	M	56
MT	1	11	9.2	3.9	0.6	L	M	60
M2	5	12	9.2	3.9	0.6	L	M	60
T	6	16	5.8	4.2	1.17	L	M	52

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

Time (months)	Patient 1	Patient 2	Patient 3	Patient 4	
				Toe	Heel
Initial	2.0 x .4x.5Q	3.8x1.3xT	6x3.5x8	.1x.8 x 5Q	1.3x.5x5Q
1	closed	3.0x1.3xT	5x1.5x8	.1x closed	.5x.5x5Q
2		3.0x1.3x5Q	4.8x1.1x5Q		1x1x5Q
3		2.5x.5x5Q	3.5x.5x0		.6x.7x0
4		.9x.1x0	closed		.7x.5x5Q
5		closed			closed

B= deep to bone; D= deep to dermis; T=deep to tendon; 5Q= deep to subcutaneous



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

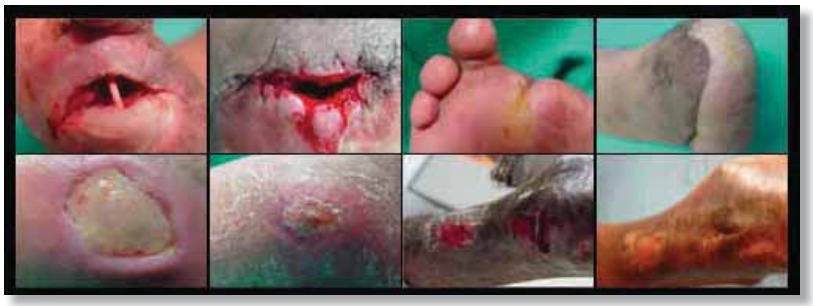
We evaluated 12 ulcers on 9 patients; 2 venous stasis, 5 post operative dehiscences, and 5 DFU's. All patients received weekly applications of Human Fibroblast-derived Dermal Substitute, topical oxygen and conventional wound care consisting of infection control, debridement, off-loading or compression. Average ulcer size was 4.6 X 1.9cm 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.



Patient	Age (Yrs)	Duration (Months)	Size (CM)	Depth (CM)	Time to Closure (Weeks)	ABI	TBI	Wound Type
1	57		2.3 X 1	1	10	R-NC and L-NC	.1 and .53	Dehiscence
2	81	6	3.4 X 2.3	1.4	16	R-NC and L-NC	.2 and .3	DFU
3	67	19	4.2 X 2.3	1	16	R-.81 and L-.71	.5 and .47	VLU
4	76	13	3.7 X 2	1.2	16	R-NC and L-NC	.23 and .41	DFU
5	70	6	4.2 X 2.7	1.2	16	R-NC and L-NC	.3 and .37	DFU
		6	2.4 X 2.1	1.2 sinus tract	14	R-NC and L-NC	.3 and .37	DFU
6	53		4.8 X .8	2.9	16	R-NC and L-NC	.52 and .74	Dehiscence
7	68	36	3.6 X 2.9	1.2	12	R-.78 and L-.6	.52 and .31	VLU
8	86	6	4.6 X 3.9	1.3	8	R-NC and L-NC	not recorded	DFU
9	69		5.2 X 2.5	1	10	R-.50 and L-.7	.24 and .28	Dehiscence
			9.3 X 2.8	1.4	15	R-.50 and L-.7	.24 and .28	Dehiscence
			3.9 X 2.9	1	7	R-.50 and L-.7	.24 and .28	Dehiscence
Avg.	70	13	4.3 X 2.3	1.3	13			

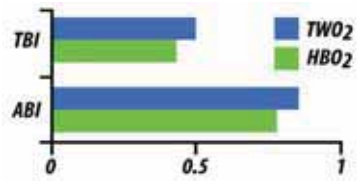
Hyperbaric and Topical Wound Oxygen: A Comparative Study

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

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

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

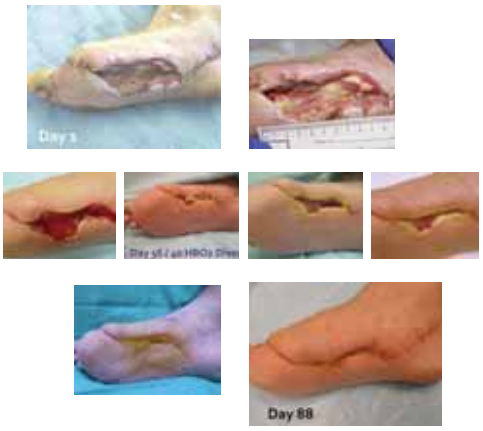
Patient Data	HBO_2	TWO_2
Age	52	64
DM	13 yrs	21 yrs
Hx of amputation	x2	x1
PMH	DM, HTN, Hep C, Cirrhosis	DM, HTN, Obesity, Kidney Dx
Smoking	20 pack yrs	Negative
ABI/TBI	.85 / .45	.90 / .52



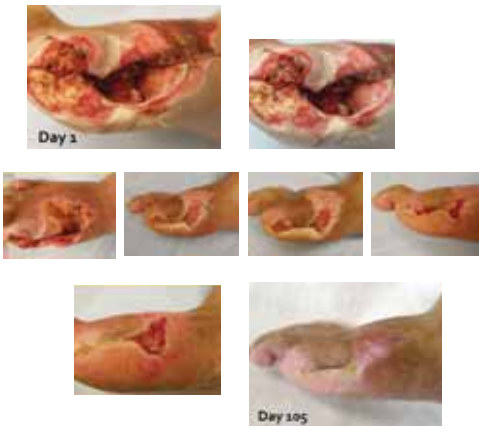
Results:

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

Hyperbaric Oxygen (HBO_2)



Topical Wound Oxygen (TWO_2)



MODALITY	Wound Size/ Depth	Negative pressure	O2 Therapy	Healing time	Cost
HBO_2	11.2 x 4.8 cm depth 2.7 cm	17 days	40 dives (56 days)	88 days	$23,160
TWO_2	11.9 x 5.4 cm depth 3.1 cm	23 days	210 applications (105 days)	105 days	$11,445

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

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

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

Introduction

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

Method

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

Results

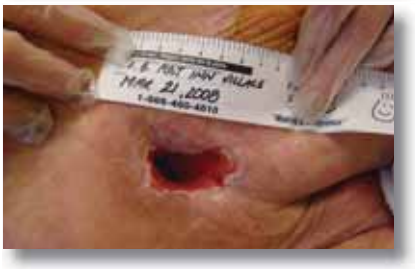
Initial wound measurements indicated the ulcer had an area of 31.2 cm2 with a volume of 109.2 cm3. Tissue was noted to be very necrotic and the peri-wound was macerated. After one week of treatment, the wound area and volume had increased slightly, however the physician noted that the maceration had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

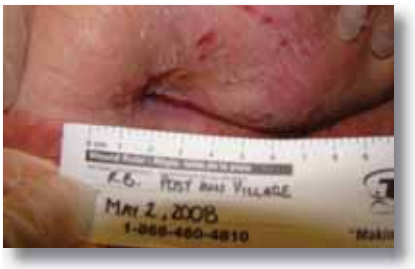
Observations:

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

Conclusion

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





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Presented at EWMA 2010 May 26-28, Geneva Switzerland

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

↓

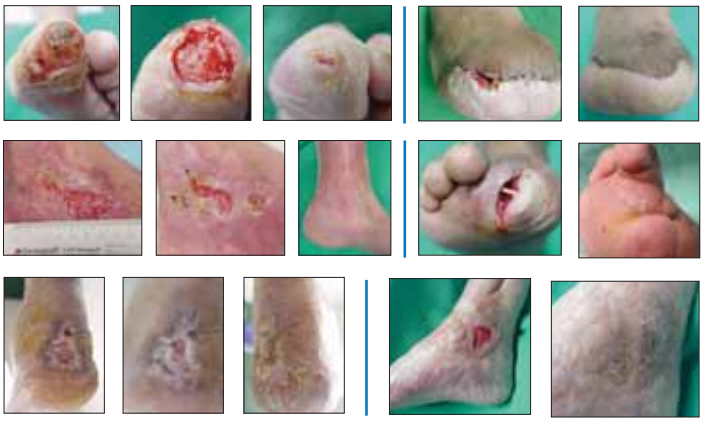
$45,000 → Amputation

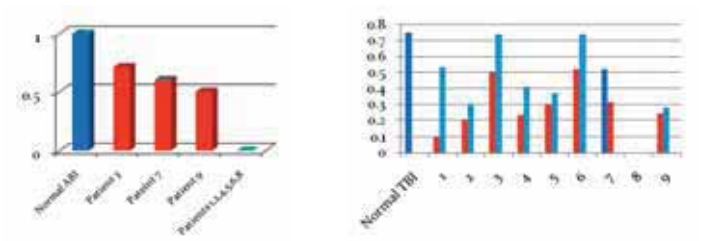
Purpose

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, offloading 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

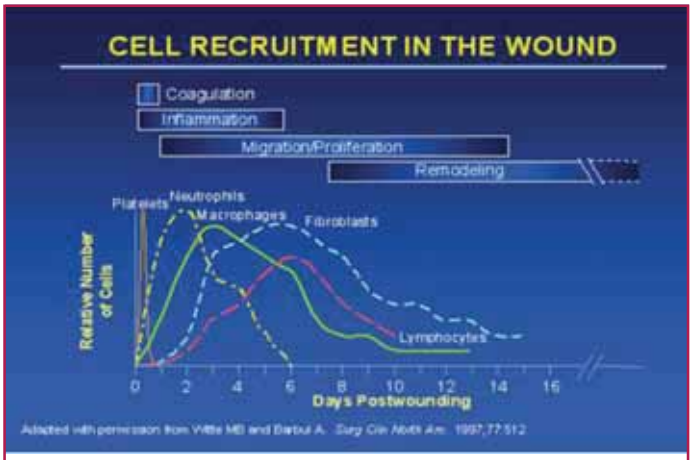
9/9 patients who were not candidates for bypass achieved closure with the combination of Topical Wound Oxygen therapy, weekly applications of HFDS, and conventional wound care consisting of infection control, debridement, off-loading or compression. The average time to closure was 12.7 weeks.

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



ANGIOGENESIS & HEALING = REQUIRES PROLIFERATION OF (1) CAPILLARY CELL WALLS (ENDOTHELIAL CELLS), (2) SUPPORTED BY COLLAGEN SCAFFOLDING (FIBROBLASTS), (3) COVERED BY EPITHELIUM (KERATINOCYTES)

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 O2-ase for ATP generation, uses 8O% of O2 breathed
  - Prolyl hydroxylase for collagen synthesis, req. for angiogenesis
  - Phagocytic O2-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!!

Chronic Wound Evaluation

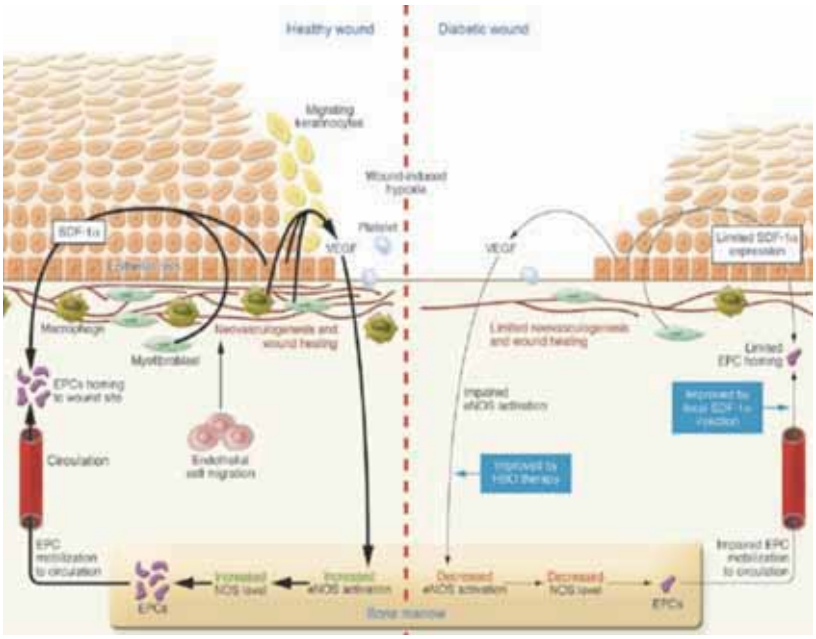
- Healing is "stalled" in chronic non-healers, typically hyper-inflammed, hyp-oxic.
- Angiogenesis, new capillary synthesis, is required for wound healing to restore blood flow (O_2 & nutrients in, waste & toxins out).
- Growth factors, secreted by platelets, neutrophils and macrophages, are required to induce angiogenesis.
- *Angiogenic biomarkers of new healing are needed:*
  - Endogenous growth factors, ie VEGF, FGF2
  - Functional neo-vessel surface marker, ie Integrin $\alpha v\beta 3$
  - Endothelial Progenitor Cell homing signal, ie SDF-1
  - Endothelial secreted vasodilator, ie Nitric Oxide

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** $\alpha v\beta 3$
- 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** $\alpha v\beta 3$**, SDF-1**

Treatments and Wound Fluid Collection

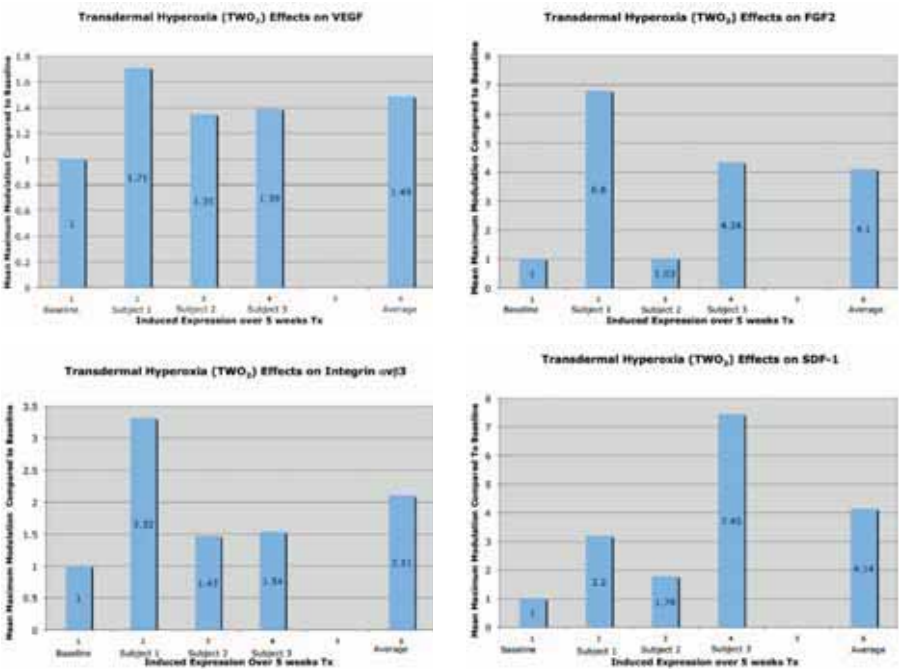
- Topical Wound Oxygen Treatments (TWO_2) were administered with medical grade oxygen (>95% pure) in a TOCE (Topical Oxygen Chamber for Extremities) for 4 consecutive days, 90 minutes per treatment for 5 weeks.
- Wounds were digitally photographed and wound fluids were collected after treatment on day 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 –20oC for subsequent assay
- Simultaneous quantification of analytes was performed using a customized multiplex enzyme-linked immunosorbent assay (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.



Physiological relevance of "new" Therapeutic Angiogenic biomarkers

Summary of Results of Therapeutic Angiogenic BioMarkers During Transdermal Hyperoxia (TWO_2) Treatments

- Angiogenic Growth Factors
  VEGF &FGF-2 increased significantly
- Integrin $\alpha V\beta 3$ (only transiently expressed in new endothelial membrane) increases correspond to angiogenic growth factors' changes
  - confirms formation of new functional capillaries and O_2 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 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 (TWO_2) demonstrates efficacy that reduces costs while improving benefits to a larger number of patients.

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

Blackman E[1], Moore C[1], Frye C[2]
[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 (TWO_2) 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 TWO_2 or advanced moist wound treatment (AMWT).

Method

TWO_2 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 TWO_2 treatment group recruited more severe wounds. The TWO_2 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 TWO_2 demonstrated significantly higher and faster healing rates with no ulcer reoccurrence after two years compared to AMWT. TWO_2 has the potential to provide substantial quality of life and cost savings benefits to both patients and the health care system as a whole.

Figure 1: Study Population

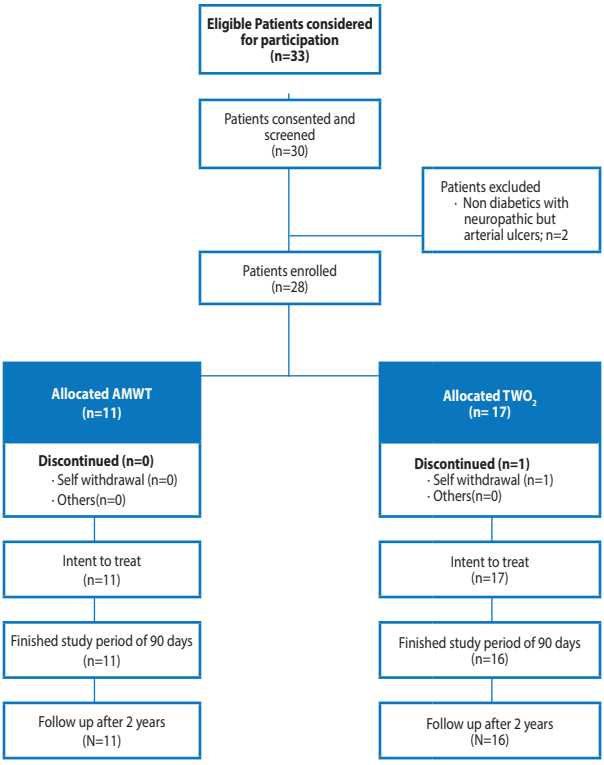
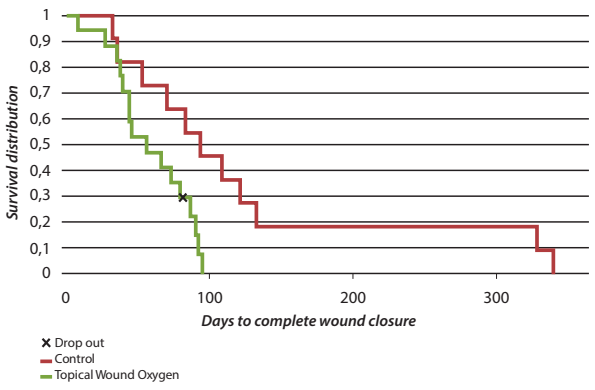


Figure 2: Kaplan-Meier estimated for time to complete wound closure



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Presented at the 5th Joint Meeting of the European Tissue Repair Society & Wound Healing Society in Limoges, France

The use of Topical Wound Oxygen (TWO_2) on Complex Recalcitrant Wounds in Multi-Morbid Patients

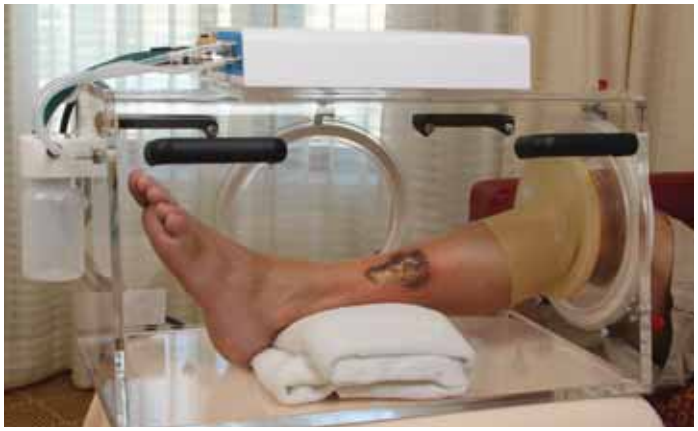
Authors: Dr. Bruce Levine[1], Dr. Christian Frye[2] 1 Harbor Foot and Ankle, San Pedro, CA 2 AOTI Ltd, Galway, Ireland

Introduction

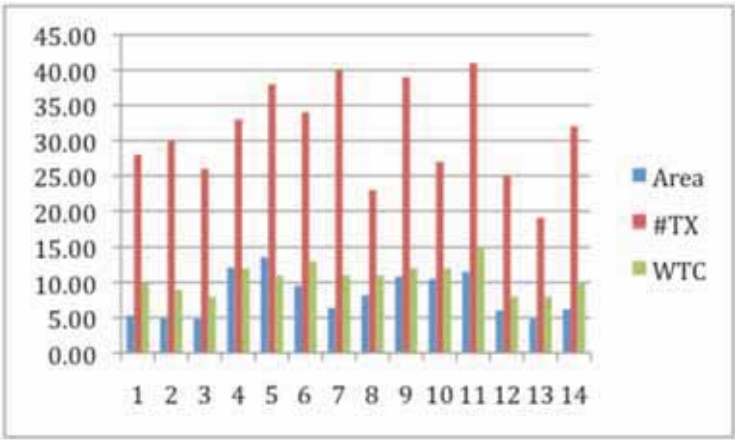
Patients suffering from chronic wounds often have multiple chronic conditions that impair wound healing.

Methods and Results

Topical Wound Oxygen (TWO_2), 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 TWO_2 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.

TopicalWound™ OXYGEN

First Experience in the Treatment of Chronic Venous Ulcers with Topical Wound Oxygen (TWO_2) in an Out-Patient setting in Latvia

Aleksandra Kuspelo

Aim

We want to share our first experience with TWO_2 in Latvia using a topical oxygen chamber using cycling pressure as an additional method in the treatment of venous ulcers

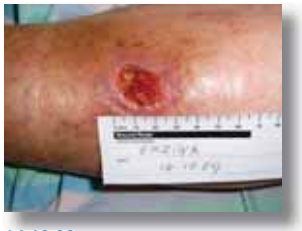
Method

The patients were treated daily with TWO_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_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.

Patient 2:



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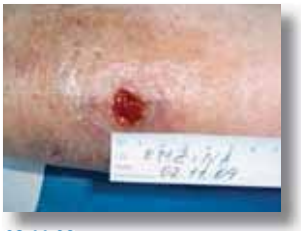

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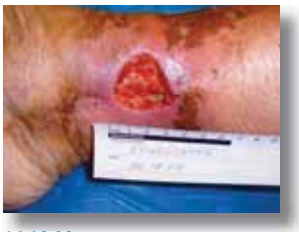
Conclusion

First experience of using TWO_2 is very positive. Patients with severe venous ulcers benefit from treatment with TWO_2 and show remarkable wound closure rates.

Patient 1:



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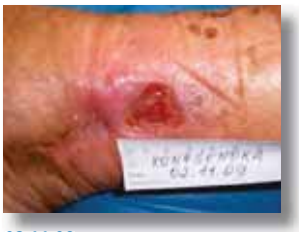

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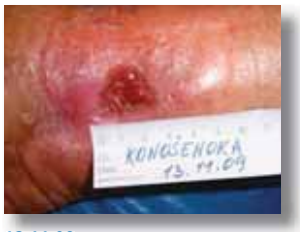

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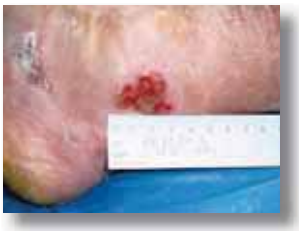
Patient 3:



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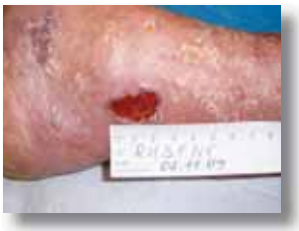

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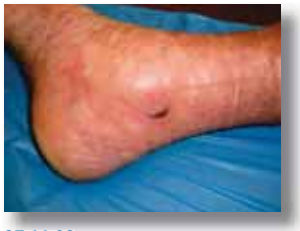

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Presented at EWMA 2010 May 26-28, Geneva Switzerland

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

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

Introduction

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

Method

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

Results

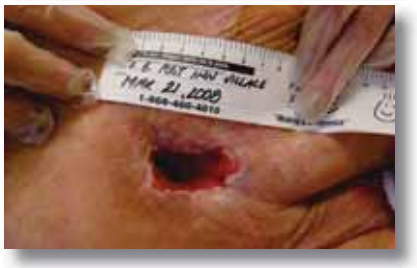
Initial wound measurements indicated the ulcer had an area of 31.2 cm2 with a volume of 109.2 cm3. Tissue was noted to be very necrotic and the peri-wound was macerated. After one week of treatment, the wound area and volume had increased slightly, however the physician noted that the maceration had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

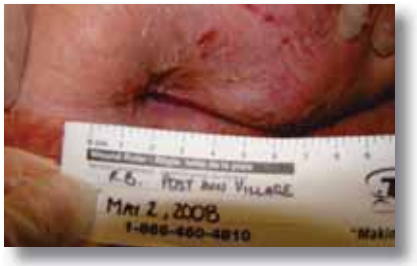
Observations:

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

Conclusion

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





Presented at EWMA 2010 May 26-28, Geneva Switzerland

TopicalWound™ OXYGEN

Therapy of a Septic Forefoot Phlegmone with Topical Wound Oxygen (TWO_2) in an Intensive Care Setting

Dr. Helmut Adler[1], Dr. Christian Frye[2]
[1] Klinikum Forchheim, Forchheim, Germany, [2] AOTI Ltd, Galway, Ireland

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 TWO_2 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 TWO_2 is well tolerated. It promotes excellent healing in complex wounds and seem to be a valuable adjunctive therapy.

Figure 1, 2: Begin with TWO_2 Therapy in Intensive Care Unit

Figure 3, 4: After the 12th day of therapy

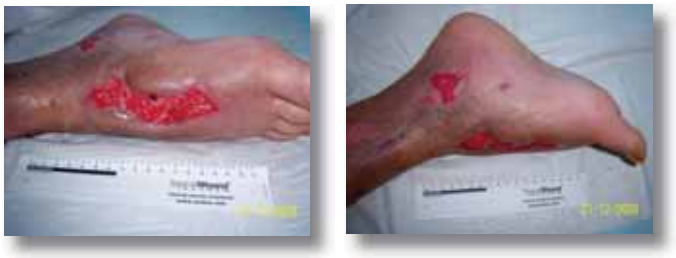


Figure 5, 6: One week after termination of TWO_2. In total 16 days of TWO_2 treatment from 3 to 6 hours daily in Intensive Care Unit.

Presented at EWMA 2010 May 26-28, Geneva Switzerland

TopicalWound™ OXYGEN

Limb Salvage with Topical Wound Oxygen (TWO_2) – Two Cases of Complex Wounds in Multimorbid Patients and Imminent Major Amputation

Dr. Helmut Adler[1], Dr. Christian Frye[2]
[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.

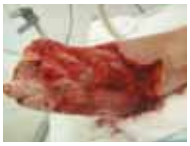
Methods and Results

Topical Wound Oxygen (TWO_2) 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:

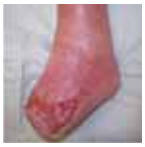
A 64 year old male patient had an autologous femoro-popliteal bypass surgery done 4 weeks prior to admission. We saw the patient with a complete necrosis of the skin on dorsal site of the foot. Surgical removal of necrosis and resection of compartment on back of foot as well as amputation of toes were performed. We continued therapy with negative pressure therapy (NPT) and intermittent TWO_2 therapy. After skin grafting NPT and intermittent TWO_2 therapy for 7 days was done. After stopping NPT, TWO_2 treatment alone was for 10 days before dismissal home.

17th July 2009. Patient presented in our hospital



Two days after surgery. Therapy with NPT and TWO_2 during dressing changes

Dismissed to home. After mesh-grafting NPT and intermittent TWO_2 therapy followed by TWO_2 therapy alone.



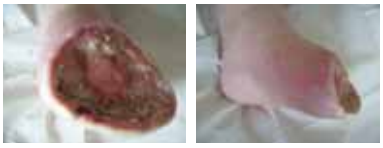
23 weeks after surgery

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 TWO_2 for 6 days. The wound granulated well and we decided to skin graft followed by negative pressure and TWO_2 therapy on days of dressing changes for 6 days. After 13 days of solely TWO_2 the wound granulated well and the patient was dismissed to rehabilitation.

2nd June 2009. Treatment of gangraenus wound since January. Transmetatarsal amputation due to worsening infection.

19th of June, NPT started.



15th of July. Increasing swelling and infection. Start with TWO_2 therapy

21/22nd July. After 6 treatments with TWO_2. Mesh grafting planned

Dismission 3 weeks after mesh-grafting. 13 days with TWO_2 therapy.

41 weeks after first admission. Readmission due to gangrene on big toe on the right foot.

Conclusion

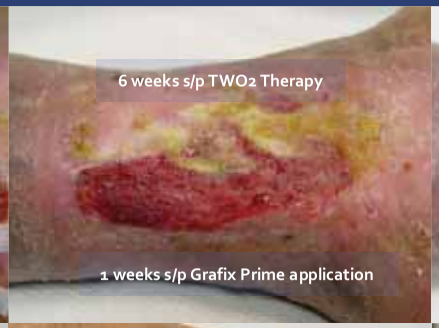
In these two complicated cases both associated with severe co-morbidities TWO_2 proved to be an valuable adjunctive therapy with good results in healing and more important to maintain the functional status by avoiding major amputation.

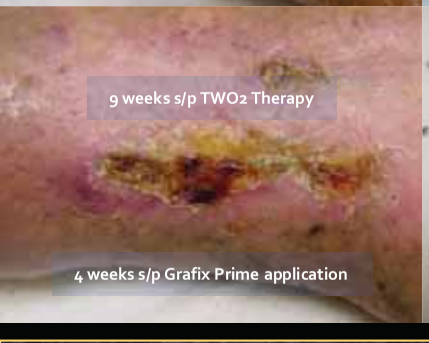
Presented at EWMA 2010 May 26-28, Geneva Switzerland

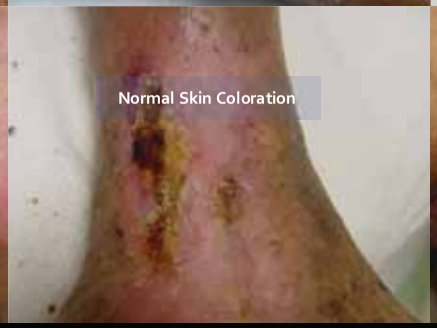
The Use of Topical Wound Oxygen Therapy (TWO2) and Grafix Prime Mesenchymal Stem Cells for Chronic Severe Venous Stasis Ulcerations

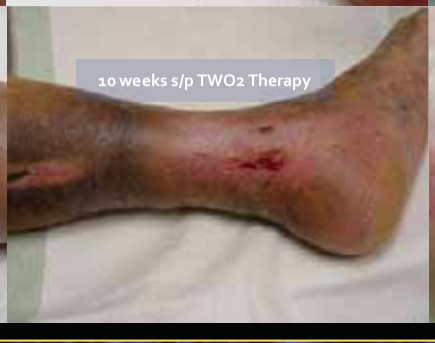
Francis Derk, DPM , CAPT, USN
STVHCS: Chief Podiatry Services
UTHSC: Adjunct Clinical Faculty

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.





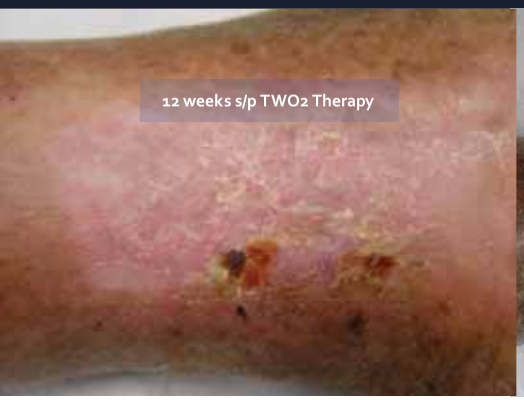


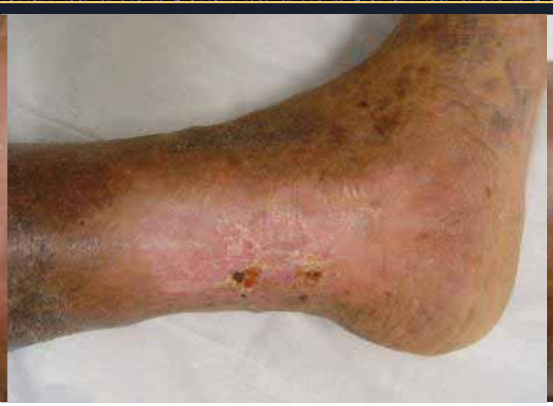


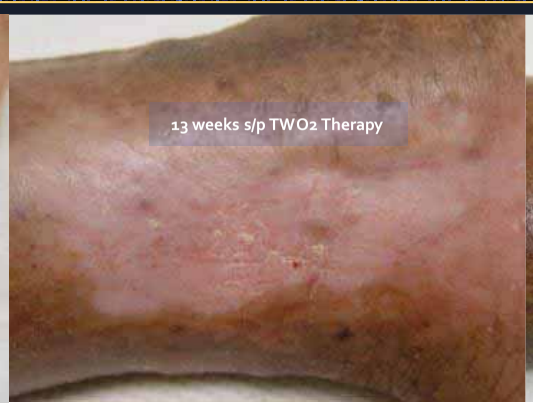
Left Leg:
Venous Stasis Ulcerations x 2
Grade IIA: 11.2 x 5.5 cms / 8 x 4 cms
Radiographs: unremarkable
WBC 6.2, Sed Rate 20, CRPH .8
ABI: Right .70 Left . 68
Venous Duplex Scan: Negative
C&S: S. aureus (negative MRSA)
Keflex 250 QID x 14 days

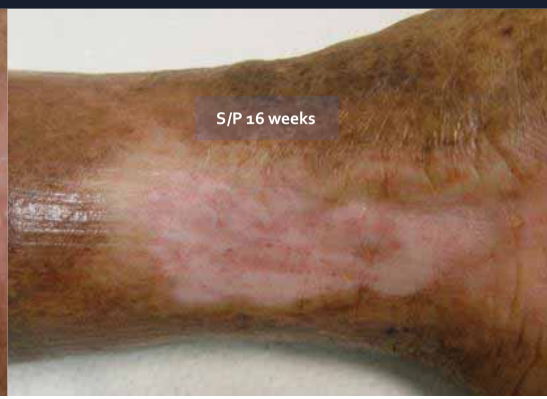
Treatment and Methodology: The 2 large stasis ulcerations c/o non-viable, dry wound bases. Topical Wound Oxygen was initiated first for 90 minute sessions bid followed by Santyl dressings. Wound Conversion took place approximately 5 weeks afterwards where the wound base became granular and Grafix Prime 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

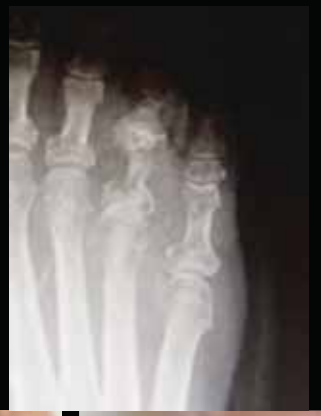
Francis Derk, DPM , CDR USN
STVHCS: Chief Podiatry Services
UTHSC: Adjunct Clinical Faculty

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

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

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)



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.

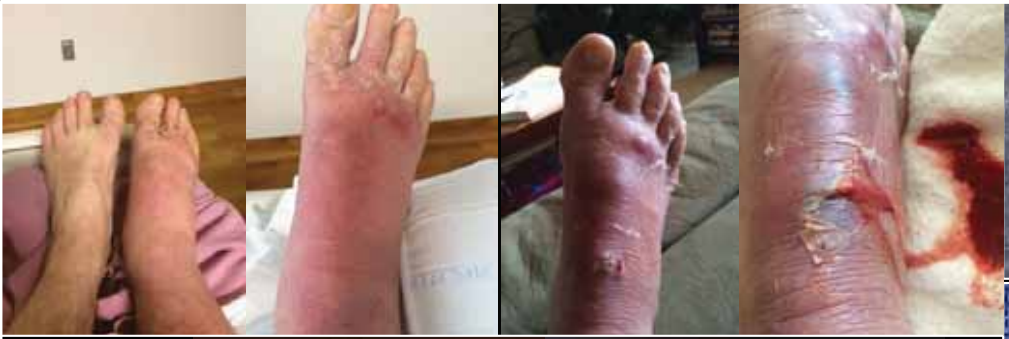
RESULTs: The CRPH level dropped to WNL at approximately week 6 and wound Conversion was attained to a UT Grade II A with full healing at week 9. Follow up X-rays and labs were unremarkable and the patients kidney function was still operable.

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

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

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.



Initial Presentation

*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 x14

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

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.

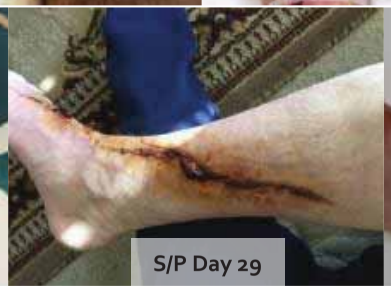
S/P Day 1: I&D of the Right Lower Extremity

S/P Day 3

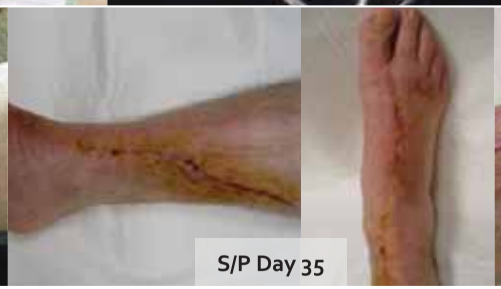
TWO2 Session immediately following D/C

S/P Day 10

S/P Day 21



S/P Day 29



S/P Day 35

S/P Day 49

PERSPECTIVE ARTICLE

Wound healing essentials: Let there be oxygen

Chandan K. Sen, PhD

The Comprehensive Wound Center, Department of Surgery and Davis Heart and Lung Research Institute, The Ohio State University Medical Center, Columbus, Ohio

Reprint requests:
Prof. Chandan K. Sen, PhD, 513 Davis Heart & Lung Research Institute, The Ohio State University Medical Center, 473 W. 12th Avenue, Columbus, OH 43210.
Tel: +1 614 247 7658;
Email: chandan.sen@osumc.edu

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

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

WOUND ISCHEMIA AND HYPOXIA

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

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

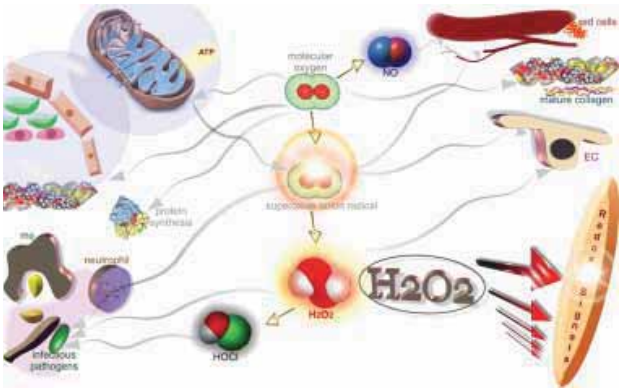

Figure 1. Significance of molecular oxygen and its derivatives in wound healing. In its molecular form, oxygen is required for oxidative metabolism-derived energy synthesis, protein synthesis, and the maturation (hydroxylation) of extracellular matrices such as collagen. Molecular oxygen is also required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

heart disease, high altitude) may contribute to wound hypoxia as well. Depending on factors such as these, it is important to recognize that wound hypoxia may range anywhere from near-anoxia to mild–modest hypoxia.[26,27] In this context, it is also important to appreciate that point measurements[28] performed in the wound tissue may not provide a complete picture of the wound tissue biology because it is likely that the magnitude of wound hypoxia is not uniformly distributed throughout the affected tissue especially in large wounds. This is most likely the case in chronic wounds presented clinically as opposed to experimental wounds, which are more controlled and homogenous in nature. In any single problem wound presented in the clinic, it is likely that there are pockets of near-anoxic as well as that of different grades of hypoxia (Figure 2). As the weakest link in the chain, tissue at the near-anoxic pockets will be vulnerable to necrosis, which in turn may propagate secondary tissue damage and infection. Pockets of extreme hypoxia may be flooded with hypoxia-inducible angiogenic factors but would fail to functionally vascularize because of insufficient O_2 that is necessary to fuel the

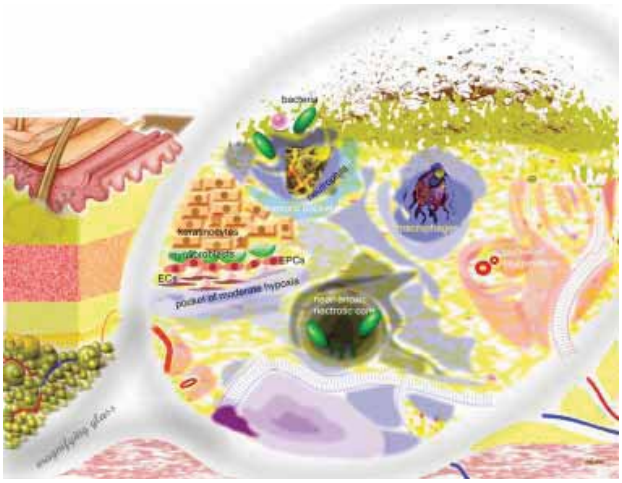

Figure 2. Heterogeneous distribution of oxygen in the wound tissue: hypothetical pockets of graded levels of hypoxia. Structures outside the illustrated magnifying glass represent the macro tissue structures. Objects under the glass represent a higher resolution. Shade of black (anoxia) or blue represents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

repair process. Indeed, uncontrolled expression of vascular endothelial growth factor (VEGF) and its receptors leads to insufficient skin angiogenesis.[29] Whether cells in the pockets of extreme hypoxia are O_2-responsive is another concern. Even if such cells may have passed the point of no return in the survival curve, correction of tissue oxygenation is likely to help clean up the dead or dying tissue[30,31] and replace the void with proliferating neighboring cells. Pockets of moderate or mild hypoxia are likely to be the point of origin of successful angiogenic response as long as other barriers such as infection and epigenetic alterations are kept to a minimum.

WOUND HYPOXIA: THE IMBALANCE BETWEEN LIMITED SUPPLY AND HIGH DEMAND

Limited supply: PVDs

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

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

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

High demand: increased demand of the healing tissue

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

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

High demand: increased production of reactive species

Phagocytic NADPH oxidases

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

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

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

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

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

Nonphagocytic NADPH oxidases

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

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

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

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

Nitric oxide (NO) synthases

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

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

THE NORMOXIC SETPOINT AND OXYGEN SENSING

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

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

Hypoxia sensing

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

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

HIF-dependent pathways

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

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

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

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

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

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

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

HIF-independent pathways

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

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

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

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

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

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

Intermittent hypoxia (IH)

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

Hyperoxia sensing

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

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

Tuning the normoxic setpoint

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

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

TISSUE OXYGENATION AND WOUND THERAPY

HIF PHD-directed wound therapeutics

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

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

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

HBO

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

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

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

Topical oxygen

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

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

SUMMARY

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

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

Richard B. Fries, William A. Wallace, Sashwati Roy, Periannan Kuppusamy, Valerie Bergdall, Gayle M. Gordillo, W. Scott Melvin, Chandan K. Sen *

Laboratory of Molecular Medicine, Dorothy M. Davis Heart and Lung Research Institute and Comprehensive Wound Center, Department of Surgery, The Ohio State University Medical Center, Columbus, OH 43210, USA

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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 hyperoxia may increase granulation tissue formation and accelerate wound contraction and secondary closure. However, HBOT is not applicable to all wound patients and may pose the risk of oxygen toxicity. Thus, the efficacy of topical oxygen treatment in an experimental setting using the pre-clinical model involving excisional dermal wound in pigs was assessed. Exposure of open dermal wounds to topical oxygen treatment increased tissue pO_2 of superficial wound tissue. Repeated treatment accelerated wound closure. Histological studies revealed that the wounds benefited from the treatment. The oxygen treated wounds showed signs of improved angiogenesis and tissue oxygenation. Topically applied pure oxygen has the potential of benefiting some wound types. Further studies testing the potential of topical oxygen in pre-clinical and clinical settings are warranted.

Keywords: Pre-clinical; Therapy; Angiogenesis; Swine

1. Introduction

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

* Corresponding author. Tel.: +1 614 247 7658; fax: +1 614 247 7818.
E-mail address: sen-1@medctr.osu.edu (C.K. Sen).

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

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

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

2. Materials and methods

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

2.1. Experimental model, wounding and treatment protocol

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

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

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

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

2.2. Wound area assessment

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

2.3. Wound-bed pO_2 measurements

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

2.4. Histology

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

2.5. Statistics

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

3. Results

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

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

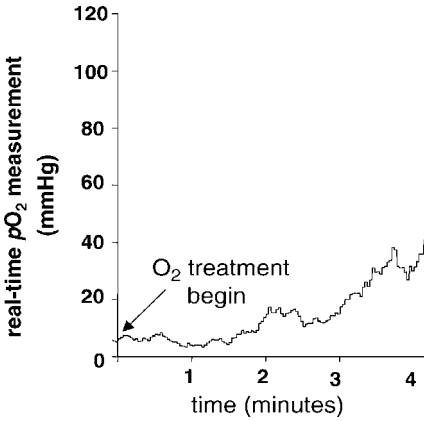

Fig. 2. Wound-bed pO_2 measurements in pigs treated or not with topical oxygen. The dermal wound model is described in this figure. pO_2 measurement was performed non-invasively using Oxy-Lite (Oxford-Optronix). An O_2 electrode was specially designed for our application (pO_2 assay at 2 mm depth) by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

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

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

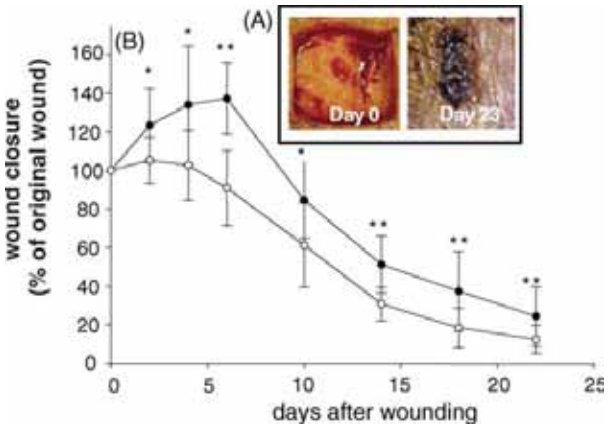

Fig. 3. Full-thickness dermal wound closure in response to topical oxygen administration in pigs. Ten (two clusters of five; on the back) secondary-intention full-thickness excisional dermal wounds (1 × 1 in.) were inflicted. Digital images of a typical wound on days 0 and 23 after wounding are shown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

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

4. Discussion

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

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

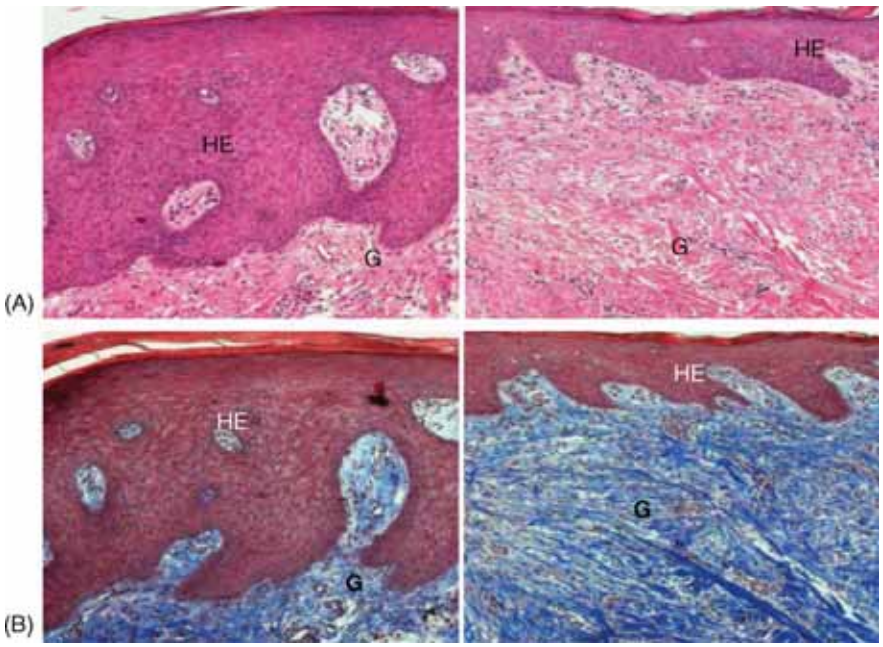

Fig. 4. Pig dermal wound histology in response to oxygen treatment. The dermal wound model is described above in Fig. 2. Three millimetres punch biopsies of the regenerated tissue were taken on day 22 from control and treated wounds. Formalin fixed paraffin sections were stained using (A) H&E or (B)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

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

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

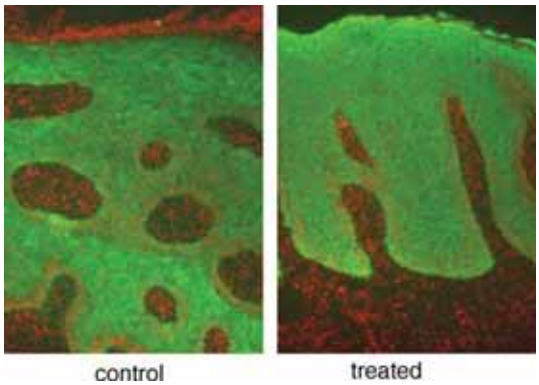

Fig. 5. Effect of oxygen treatment on epidermal remodeling during the healing process. The dermal wound model is described above in Fig. 2. Three millimetres punch biopsies were taken on day 15 from control and treated wounds. Formalin fixed paraffin sections were stained using antibody against keratin-14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

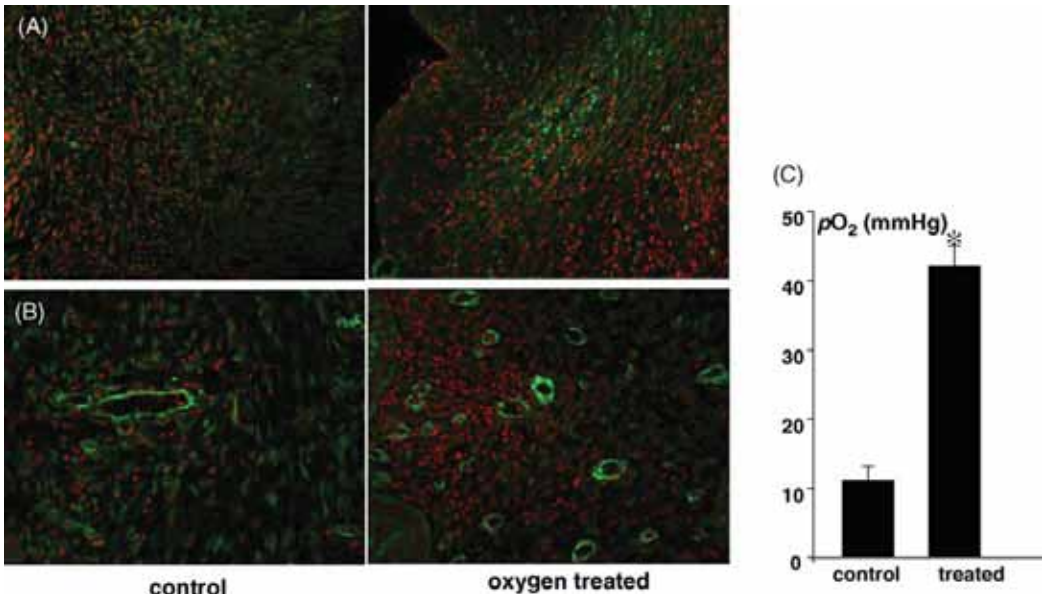

Fig. 6. Angiogenic response at the wound site following topical oxygen treatment of full-thickness dermal wounds. The dermal wound model is described above in Fig. 2. Three mm punch biopsy from wound margins were harvested. Formalin-fixed paraffin sections were stained using antibody against (A) vascular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

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

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

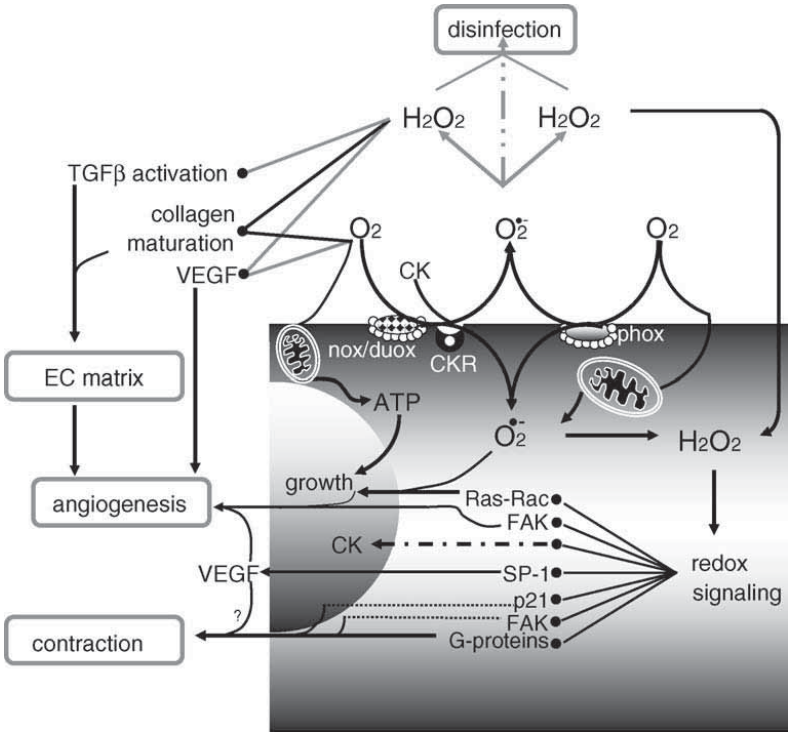

Fig. 7. Schematic illustration of select possible pathways by which oxygen and its reactive derivatives may influence wound healing related processes. The specific processes have been recently reviewed [43]. Excess generation of ROS, such as in cases where the inflammatory phase is not resolved in a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

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

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

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