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Review

TWO2 Therapy Demonstrates Superior Long-Term Outcomes Compared to Other Advanced Wound Care Modalities: Real World Evidence Supported by Mechanistic and RCT Clinical Data

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Abstract

Background and Objectives: Chronic diabetic foot ulcers (DFUs) and venous leg ulcers (VLUs) remain a major source of morbidity, healthcare utilization, and limb loss, despite adherence to established standards of care protocols and the widespread availability of advanced wound technologies. Many advanced modalities only target isolated aspects of wound healing and fail to address the complex, interdependent pathophysiology of chronic wounds, particularly tissue hypoxia, edema, impaired microcirculation, and persistent inflammation. Cyclical Pressurized Topical Wound Oxygen (TWO2) therapy is a home-based, multimodal intervention that combines humidified topical oxygen delivery with cyclical non-contact compression to address these core drivers simultaneously. *Materials and Methods*: This review synthesizes mechanistic rationale and evidence from randomized controlled trials, long-term venous ulcer studies, and real-world comparative effectiveness analyses. Emphasis is placed on the large cohort study by Yellin et al., which directly compared TWO2 with other advanced modalities including negative pressure wound therapy (NPWT), skin substitutes, and growth factor therapies. *Results*: Across these studies, TWO2 therapy is consistently associated with improved healing durability, reduced recurrence, and substantial reductions in hospitalization and amputation rates compared with both standard care and advanced wound therapies. *Conclusions*: The convergence of randomized and real-world evidence supports TWO2 therapy as a clinically meaningful and mechanism-driven adjunctive treatment option for patients with chronic, high-risk lower-extremity wounds.

Keywords: chronic wounds; topical oxygen therapy; intermittent compression; Cyclical Pressurized Topical Wound Oxygen (TWO2); multi-modality intermittent topical oxygen therapy (ITOT); inflammation resolution; wound healing; advanced wound care

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1. Introduction

Chronic lower-extremity wounds represent a growing global health challenge, driven by the increasing prevalence of diabetes, vascular disease, and advanced age.[1] Diabetic foot ulcers (DFUs) and Venous leg ulcers (VLUs) are among the most challenging to manage, frequently resulting in serious complications such as infection, hospitalization, and amputation. These adverse outcomes contribute significantly to morbidity, mortality, and healthcare costs.[2–5] Despite the availability of numerous advanced wound care technologies, real-world outcomes remain suboptimal. Many wounds fail to progress beyond the inflammatory phase of healing, even when treated with advanced therapies, such as negative pressure wound therapy, bioengineered skin substitutes, or topical growth factors. A common limitation of these approaches is their inability to adequately correct the chronic wound microenvironment, which is characterized by hypoxia, edema, impaired microcirculation, and excessive protease activity.[6] Cyclically pressurized topical wound oxygen (TWO2) therapy, has emerged as a promising adjunctive treatment modality capable of directly addressing oxygen deficiency while concurrently reducing edema, improving perfusion, and modulating inflammation.

This article synthesizes findings from randomized and real-world studies, contextualizes outcomes against other advanced treatments, and integrates mechanistic insights that provide the foundation for the superior long-term healing outcomes seen with TWO_2 therapy.

2. Materials and Methods

2.1. Pathophysiologic Rationale for Oxygen-Based Multimodal Therapy

Chronic wounds differ fundamentally from acute wounds in both biology and behavior. Rather than progressing through the orderly phases of healing, chronic wounds remain trapped in a state of persistent inflammation.[7] Tissue hypoxia is a defining feature of this state, resulting from macrovascular disease, microvascular dysfunction, edema-related capillary compression, impaired oxygen diffusion, and inflammatory metabolic demand.[8]

Hypoxia disrupts multiple healing pathways. Fibroblast proliferation and collagen deposition are oxygen-dependent processes, as is angiogenesis. Immune function is similarly compromised, reducing bacterial killing, and increasing the risk of infection.[8]

Edema increases interstitial pressure, compresses capillaries, and lengthens oxygen diffusion distance, further amplifying tissue hypoxia. Inflammatory mediators and proteases accumulate due to inadequate lymphatic clearance, degrading growth factors and extracellular matrix components.[9,10] Repetitive ischemia–reperfusion injury generates oxidative stress, further impairing tissue repair.[6]

Effective chronic wound therapy must therefore address oxygen delivery, fluid dynamics, edema, and inflammation in an integrated manner. Therapies that target only wound size, exudate, or surface coverage may fail to produce durable healing if these underlying drivers are not corrected.

2.2. The Synergy of Topical Oxygen, Cyclical Compression and Humidification

Cyclical Pressurized Topical Wound Oxygen therapy was developed to address the multifactorial pathophysiology of chronic wounds through a single, integrated system. The therapy combines three synergistic components: topical oxygen delivery, cyclical non-contact compression, and humidification.

Topical oxygen delivery increases tissue oxygen tension at the wound surface, supporting oxidative bacterial killing, collagen synthesis, and angiogenesis.[8,11–16] Unlike systemic oxygen approaches, topical delivery targets the wound directly, minimizing systemic exposure while maximizing local effect.[12]

Therapeutic level cyclical compression augments these effects by increasing the oxygen partial pressure delivered to the wound bed, facilitating lymphatic clearance of inflammatory mediators,

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reducing edema, restoring microvascular pressure gradients, and improving oxygen diffusion into hypoxic tissue.[6] At the cellular level, cyclical mechanical deformation activates endothelial and stromal mechanotransduction pathways, including eNOS and integrin-mediated signaling, that promote inflammation resolution, angiogenesis, fibroblast proliferation, extracellular matrix deposition, and collagen cross-linking.[6] (Figure 1)

Humidification improves oxygen diffusion, maintains a moist wound environment, and supports organized tissue formation and durable healing.[17] Together, these mechanisms promote a biologically favorable wound environment that supports not only closure but also durable tissue repair.[6]

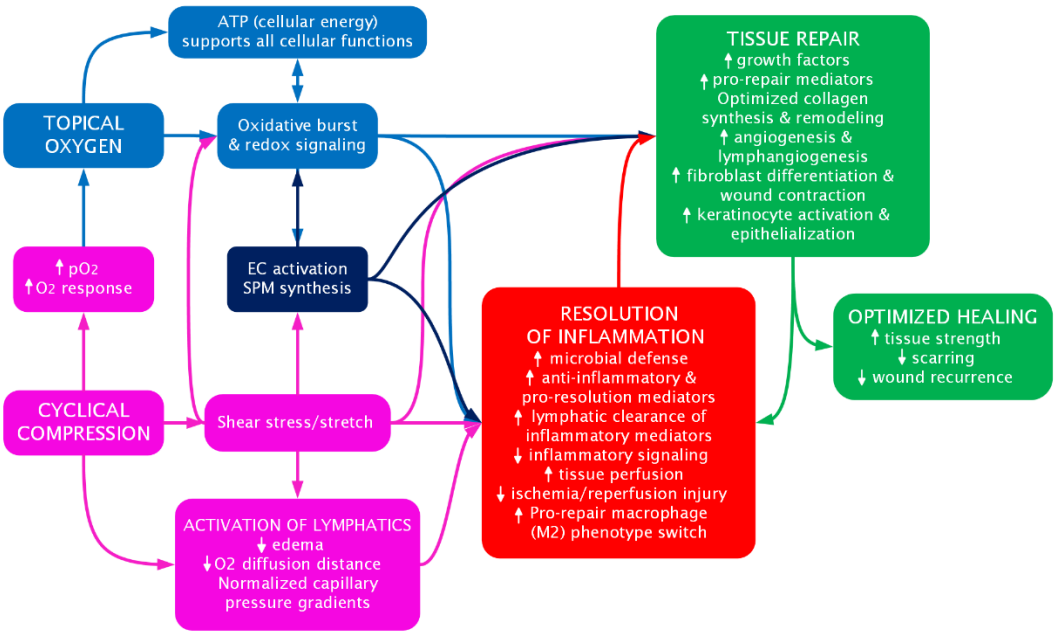

Figure 1. TWO2 Mechanism of Action: The integrated cellular and molecular mechanisms by which topical oxygen and cyclical compression synergistically promote wound healing. Topical oxygen increases tissue oxygen tension to fuel ATP production, enhance microbial defense via oxidative burst, activate redox signaling, and optimize collagen synthesis and crosslinking. Cyclical compression increases the partial pressure (pO_2) of topical oxygen and activates lymphatic function, improving clearance of inflammatory mediators, reducing edema, decreasing diffusion distance for oxygen, normalizing capillary pressure gradients, and restoring perfusion. Compression-induced shear stress and shear stretch activate endothelial cells (ECs) and stimulate the biosynthesis of specialized pro-resolving mediators (SPMs). This initiates a cascade of anti-inflammatory and pro-resolution signaling, including polarization of macrophages toward the reparative M2 phenotype and acceleration of inflammation resolution. In parallel, M2 macrophages, SPMs, and ECs upregulate growth factors and reparative cytokines that direct wound repair and remodeling. Activated ECs stimulate angiogenesis and lymphatic angiogenesis, while fibroblasts drive collagen synthesis, ECM production, and myofibroblast differentiation, enabling wound contraction. Keratinocyte activation promotes epithelialization, and during remodeling, fibroblast activity enhances collagen fiber organization. Collectively, these pathways promote efficient resolution of inflammation, improved perfusion, increased tissue strength, reduced scarring, and lower wound recurrence. pO2, partial pressure of oxygen; EC, endothelial cell; SPM, specialized pro-resolving lipid mediators; NADPH, nicotinamide adenine dinucleotide phosphate; ATP, adenosine triphosphate; M2, pro-repair macrophage phenotype. (Illustration by Blakely MM, 2025). Reprinted from: Lohr JM, Raffetto JD, Dexter DJ, et al. A synergistic multimodality treatment approach to address the key drivers of wound chronicity. Journal of Vascular Surgery: Venous and Lymphatic Disorders. 2026;14(1):102348. doi:10.1016/j.jvsv.2025.102348[6].

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3. Results

3.1. Evidence from Randomized Controlled Trials

High-quality randomized evidence supporting TWO2 therapy is provided by the multicenter, double-blind, sham-controlled TWO2 Study conducted in patients with chronic DFUs that had failed to respond to standard care and published in the leading Diabetes Care journal.[18] Participants were randomized to receive either active TWO2 therapy or sham treatment in addition to optimal wound care.

At the first prespecified interim analysis, healing at 12 weeks was significantly higher in the active TWO2 group compared with sham (41.7% versus 13.5%), with an adjusted odds ratio of 6.00 (97.8% CI 1.44, 24.93), p = 0.004, after controlling for ulcer severity. Time-to-healing analysis demonstrated a more than fourfold greater likelihood of healing over 12 weeks in the TWO2 group (HR = 4.66 [97.8% CI 1.36, 15.98], p = 0.004).

Uniquely and importantly, durability of healing was confirmed at one year, with a significant 56% of ulcers in the active group remaining closed compared with just 27% in the sham group (p = 0.013). This sustained healing at one year suggests improved tissue remodeling, which was further demonstrated by a six-fold reduced ulcer recurrence.

3.2. Evidence in Venous Leg Ulcers

In a prospective controlled study of 132 patients with chronic VLUs present for more than two years, TWO2 therapy was compared to conventional compression dressings. [19,20] Key and statistically significant findings:

- Healing rate: 76% vs 46% at 12 weeks (P < 0.0001)
- Median time to closure: 57 vs 107 days (P < 0.0001)
- Recurrence at 36 months: 6% vs 47% (P < 0.0001)

Pain reduction and improved infection resolution were also observed in the TWO2 group. These findings extend the relevance of TWO2 beyond diabetic wounds to those with venous pathology, where edema and hypoxia are central features. This is particularly important as most patients treated in the real world are comorbid with multiple etiological traits.

3.3. Real-World Evidence in Chronic Lower Extremity Wounds

While randomized trials establish efficacy, real-world evidence is critical to understanding clinical value across heterogeneous, high-risk populations. A recent large cohort retrospective study by Lohr, et. al., evaluated the effectiveness of TWO2 in chronic lower extremity wounds of varying etiologies.[21] In this group of 3126 patients, 64.8% (n=2027) achieved complete healing in 4.2 (SD±2.5) months, despite a mean pre-treatment wound age of 7 (±15.9) months. The need for retreatment due to wound recurrence was only 2.7% (n=54), with a mean follow up time of 13.9 (±4.9) months and the rates of hospitalization and amputation were 3.7% (n=115) and 6.1% (n=191) respectively, substantially lower than historical standards.[1,22–24]

Subgroup analyses demonstrated healing rates of 63.3% in a mean time of 4.2 (±2.5) months in DFUs, 72.1% in a mean time of 4 (±2.6) months in VLUs, 59.3% in a mean time of 3.9 (±2.5) months in arterial ulcers, and 65.6% in a mean time of 4 (±2.3) months in atypical wounds. Compared with large wound registry data, the more medically complex patients in the TWO2 study had higher rates of healing in both DFU and VLU (63% at 18 weeks vs. 45% at 20 weeks for DFU, and 72% at 17 weeks vs. 57% at 16 weeks for VLU).[25] The findings of this large study further support the efficacy of TWO2 in chronic wounds of varied etiologies.

3.4. Real-World Comparative Effectiveness Evidence

Comparative effectiveness evidence is crucial to evidence-based clinical decision-making, empowering clinicians and patients to select treatments most likely to produce meaningful outcomes, and enabling health systems to direct resources toward interventions that demonstrate durable value.

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A retrospective cohort analysis of 202 patients by Yellin *et al.*, evaluated the effectiveness of home-based TWO2 therapy compared to other advanced therapies in patients with DFUs treated at two U.S. Veterans Affairs hospitals.[26]

3.3.1. TWO2 vs No TWO2 (Additive Model)

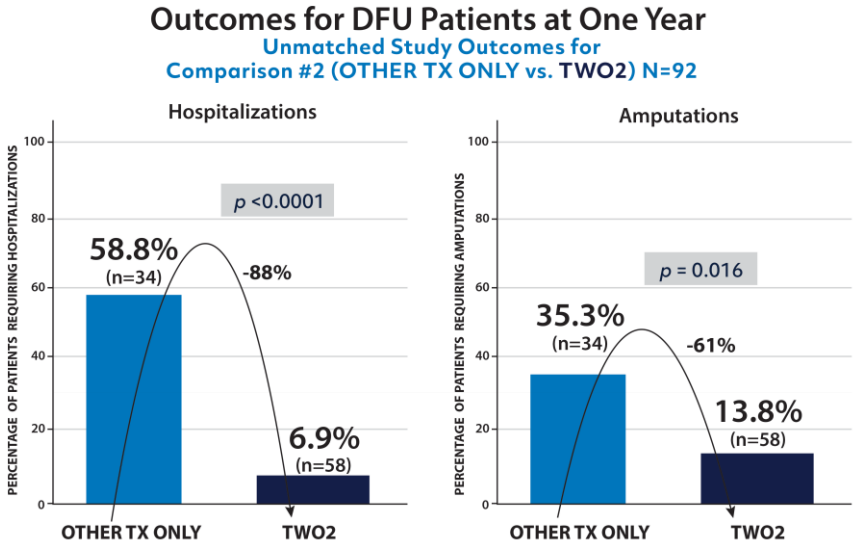
In this comparison, outcomes in patients who had received TWO2 therapy at any point during their care pathway (TWO2) versus those who had not received TWO2 therapy (NO TWO2) were evaluated. Patients in both groups may have received other additional advanced treatment interventions, therefore TWO2 therapy was considered an additive adjunctive treatment.

In unmatched cohorts, patients treated with TWO2 experienced 88% fewer hospitalizations (6.6% vs. 54.1%, $p < 0.0001$) and 71% fewer amputations (12.1% vs. 41.4%, $p < 0.0001$) over one year when compared with patients who did not receive TWO2. After propensity score matching for age, wound severity, comorbidities, prior amputation, and use of other advanced therapies, TWO2 therapy was associated with an 82% reduction in hospitalizations (7.1% vs. 40.0%, $p < 0.0001$) and a 73% reduction in amputations (8.6% vs. 31.4%, $p = 0.0007$), compared with no TWO2. Logistic regression demonstrated an almost ninefold greater risk of hospitalization and fivefold greater risk of amputation among patients who did not receive TWO2. These reductions persisted across Wagner grades and in patients with peripheral arterial disease, prior amputation, with ESRD and even on dialysis.

3.3.2. TWO2-Only vs Other Advanced Modalities (Direct Either/Or Comparison)

A unique and clinically relevant aspect of the Yellin *et al.*, analysis was a second comparison that directly evaluated TWO2-only against other advanced therapies (NPWT, skin substitutes, growth factors).

Patients treated with TWO2 alone experienced significantly better outcomes than those treated with only other advanced modalities, demonstrating an 88% relative reduction in hospitalization (6.9% vs 58.8%, $p < 0.0001$) and 61% fewer amputations (13.8% vs 35.3%, $p = 0.016$) at one year. (Figure 2)



	OTHER TX ONLY	TWO2
Yes N:	20	4
No N:	14	54
Total N:	34	58

	OTHER TX ONLY	TWO2
Yes N:	12	8
No N:	65	50
Total N:	111	58

Figure 2. TWO2-Only vs Other Advanced Modalities: One Year Outcomes: Patients treated with TWO2 alone experienced significantly better outcomes than those treated with only other advanced modalities, demonstrating an 88% relative reduction in hospitalization and 61% fewer amputations.

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These outcomes suggest that TWO2 therapy not only offers additive benefit but may also outperform other traditionally used advanced wound care modalities when used as a primary adjunctive therapy.

In whole, the study demonstrated that the use of TWO2 therapy, with or without other adjunctive treatments, was associated with significantly reduced frequency of wound-related hospitalization and amputation for patients afflicted with DFUs. Given that both outcomes are associated with higher costs, increased healthcare utilization, and poorer quality of life, these findings highlight TWO2 therapy's potential value as a cost-effective, patient-centered modality in DFU management.[2–4]

4. Discussion

4.1. Contextualizing TWO2 Amongst Other Advanced Wound Technologies

Negative pressure wound therapy is effective for managing exudate and promoting granulation tissue formation but does not directly correct hypoxia or microvascular dysfunction or reduce edema. Skin substitutes and cellular therapies depend on a viable, well-oxygenated wound bed and may fail in hypoxic or protease-rich environments. Growth factor therapies are similarly limited by rapid degradation and reduced activity under hypoxic conditions. Continuous low-flow topical oxygen systems provide minimal oxygen supplementation but lack the cyclical compression necessary to meaningfully reduce edema, improve perfusion and restore lymphatic function. A comparison of advanced wound modalities is shown in Table 1.

The comparative outcomes observed with TWO2 therapy support a mechanistic explanation: therapies that address only one component of chronic wound pathology may improve intermediate endpoints, whereas therapies that correct multiple interdependent drivers of wound chronicity are more likely to produce durable clinical results.

Table 1. Relative Comparison of Advanced Wound Therapies: Relative effects are qualitative and reflect dominant direct mechanisms reported in published mechanistic and clinical literature. "+++" denotes primary, robust engagement of the pathway; "++" consistent secondary contribution; "+" indirect or context-dependent contribution; "–" no primary direct mechanism. Ratings are comparative and not absolute measures of clinical efficacy. *Indirect effects mediated via perfusion or edema modulation rather than direct oxygen delivery or antimicrobial action.

Therapy	pO2 Increase	Edema Reduction/ Lymphatic Activation	Inflammation Resolution	Angiogenesis Upregulation	Bioburden Control	ECM/ Cellular Repair	Unique Mechanism(s)
TWO2[6,18]	+++	+++	+++	+++	++/+++	+++	Pressurized topical oxygen with non-contact cyclical compression; concurrent modulation of hypoxia, edema, and mechanotransduction signaling pathways
HBOT[27]	+++	+*	++	++	++	++	Systemic hyperoxygenation increasing dissolved plasma oxygen and tissue oxygen gradients
Continuous Delivery Oxygen[28,29]	++	-	++	++	++	++	Sustained low-flow topical oxygen diffusion maintaining continuous wound surface oxygen gradient
NPWT[30]	+*	+	++	++	+	++	Macro/microdeformation induced mechanotransduction with controlled exudate

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							removal and wound edge stabilization
Skin Substitutes[31]	-	-	+	++	-	+++	Bioactive extracellular matrix scaffold providing structural support, cellular signaling, and growth factor modulation
Growth Factors[32]	-	-	+	++	-	++	Exogenous peptide-mediated activation of cell proliferation and angiogenic signaling pathways
Compression[33,34]	+*	+++	++	+	+	+	Venous hypertension reduction and lymphatic unloading through sustained, graduated external pressure

4.2. Practical Considerations and Clinical Integration

TWO2 therapy is designed for home-based administration by the patient, reducing treatment burden and improving adherence, while advancing access to care. It can be integrated into care pathways either as an adjunct to other advanced modalities or as an alternative when other therapies fail. Sequential strategies may include initial NPWT for exudate control followed by transition to TWO2 for angiogenesis and epithelialization, or concurrent use with skin substitutes to optimize graft uptake.

4.3. Cost-Effectiveness Considerations

Wound recurrence, hospitalizations and amputations represent major cost drivers in chronic wound care. Repeat emergency department admissions, surgical intervention, rehabilitation, prosthetics, and long-term disability contribute to the economic burden. Not to mention the negative psychosocial and quality of life impacts on patients and caregivers.

The reductions in hospitalization (up to 88%) and amputation (up to 73%) observed in real-world TWO2 cohorts suggest significant potential downstream cost savings. Additionally:

- Home-based therapy reduces outpatient visit frequency.
- Improved durability should reduce recurrence-related costs.
- Lower amputation rates should reduce lifetime disability expenditures.

A recent Markov model analysis demonstrated that TWO2 was cost-dominant compared with standard care, with lower total two-year costs and improved quality-adjusted life years (QALYs).[2]

4.4. Limitations

The evidence base for TWO2 therapy includes both randomized and observational data, each with inherent limitations. Even though largely compensated for by robust study designs and statistical approaches such as propensity scoring, retrospective analyses may be subject to unmeasured confounding, while randomized trials may not fully capture real-world complexity. Direct head-to-head randomized comparisons with NPWT or HBOT are currently lacking. However, the consistency of findings and long-term outcomes across study designs and wound types strengthens confidence in the observed therapeutic benefits.

5. Conclusions

Cyclical Pressurized Topical Wound Oxygen therapy is supported by a converging body of evidence demonstrating improved healing durability and substantial reductions in hospitalization and amputation in patients with multimorbid chronic lower-extremity wounds. The award winning randomized TWO2 Study establishes efficacy, while the real-world analysis by Yellin and colleagues demonstrates generalizability in far broader comorbid patient populations, as well as comparative

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effectiveness against other advanced wound modalities. By addressing hypoxia, edema, and impaired microcirculation simultaneously, TWO2 therapy offers a mechanism-driven approach that translates into meaningful patient-centered outcomes.[6,18,19,26]

These findings support the earlier and broader adoption of TWO2 therapy in the management of chronic lower extremity wounds, with the potential to reduce wound recurrence, lower amputation risk, and decrease the economic burden and social toll of chronic wounds on patients, caregivers, and the healthcare system.

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Conflicts of Interest: MR, CD and SR have received honoraria for speaking and consulting with AOTI, Inc. CD was an investigator in the TWO2 RCT[18] and received research support from AOTI, Inc. during the conduct of the study. MG is the Chief Medical Officer of AOTI, Inc. NA and AD declare no conflicts of interest.

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