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ORIGINAL ARTICLE

Ozone therapy for the treatment of chronic wounds: A systematic review

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Chronic wounds present a significant burden to the health care system and the patient. Ozone therapy has been proposed as a treatment for chronic wounds, potentially acting by eliciting mild oxidative stress or disinfection. The purpose of this systematic review is to evaluate the potential benefits and harms of ozone therapy as an advanced care intervention for chronic wounds. Studies were extracted from Google Scholar, PubMed, the Cochrane Library, and reference lists. General inclusion criteria included English-language randomised human trials reporting the use of ozone therapy in the topical treatment of chronic wounds. Primary outcome data included the extent of chronic wound healing, and secondary outcomes included adverse effects. Studies were assessed for level of bias and data quality. Nine studies ($n = 453$ patients) matched the inclusion criteria and underwent meta-analysis. Overall, there was a significant improvement in wound closure with ozone therapy. Results consistently favour the application of ozone as a treatment for chronic wounds; however, there is no conclusive evidence of ozone therapy as superior compared with standard treatments. Compared with standard care, ozone therapy as an advanced wound care treatment may improve the proportion of chronic wounds healed in a shorter amount of time, but further research is required.

KEYWORDS
chronic wound, healing, ozone, ozone therapy, ulcer

1 | INTRODUCTION

1.1 | Chronic wounds

The healing of wounds follows a typical progression through 3 primary phases: inflammation, proliferation, and maturation.[1] Regulatory polypeptides, including transforming growth factor-β (TGF-β),[2] platelet-derived growth factor (PDGF),[3] vascular endothelial growth factor (VEGF),[4] and fibroblast growth factors (FGF),[5] are vitally important to control the stages of the healing process. However, comobidities that lead to neuropathies, ischaemia, high presence of foreign materials, and infection can reduce and damage these growth factors, ultimately inhibiting the proliferative phase.[6,7] This impairment of the healing process may result in a chronic wound. Alternatively, chronic wounds may, in part, be caused by the creation of a microbial biofilm resistant to antibiotics that prolongs the inflammatory phase.[8]

1.2 | Skin response to environmental stress

The skin is the largest organ of the body and serves as a protective covering essential for homeostasis.[9] The skin is constantly subjected to environmental stressors, including reactive oxygen species (ROS) from both endogenous and exogenous sources.[10] However, not only does the skin provide a physical barrier to these insults, it has immunological and antioxidant components as well.[11] This antioxidant defence comprises of antioxidants such as superoxide dismutase; catalases; glutathione peroxidase; and non-enzymatic low-molecular weight

antioxidants such as vitamin E isoforms, vitamin C, glutathione (GSH), uric acid, and ubiquinol.[12]

1.3 | Ozone therapy

Ozone (O_3) has been acknowledged as a potent antimicrobial agent since the 1800s.[13] Evidence supports ozone as an advanced clinical therapeutic agent for the treatment of chronic wounds, including ulcers, with significant improvements in healing outcomes.[14] The suggested mechanisms of therapeutic improvement is ozone's ability to elicit mild oxidative stress and act as a powerful disinfectant[15,16]; ozone causes irreversible damage to viral DNA[17] and bacterial cell walls[18] by oxidising the lipoproteins and phospholipids of the pathogens. Furthermore, as O_3 decomposes in blood, the free radicals readily form ROS. These ROS—including superoxide anion radical (O_2^-), hydroxyl radical (HO), and nitric oxide (NO)—can act as powerful physiological mediators for adaption by acting as vasodilators and stimulating important endogenous growth factors that can be medically beneficial.[17] However, the level of ROS must be kept within certain limits to avoid toxicity.[15,19]

1.4 | Skin cell response to ozone exposure

Reservations regarding ozone therapy stems from its toxicity to tissues, especially within the respiratory tract if administered in highly concentrated doses.[20] Studies that analysed the effect of ozone exposure to the skin of mice showed that the ozone depletes the skin's antioxidant levels and increases lipid peroxidation.[21] Toxic concentrations of ozone exposure in mice cause modification and/or oxidation of lipid and protein constituents of the epidermis.[22] However, in controlled doses, this environmental challenge can accelerate the cell cycle and induce synthesis of growth factors by activation of redox transcription factors such as nuclear factor kappa B (NFkB).[23] NFkB is an activator for proinflammatory genes interleukin 8 (IL-8), Tumour necrosis factor α (TNFα), and TGF-β and, as such, is a regulator for inflammatory responses and entire wound healing.[24]

1.5 | Application of ozone therapy

The delivery method of ozone traditionally takes 1 of 3 forms: gaseous ozone exposure within a hyperbaric chamber, ozonated oils, and ozonated water.[25] Further experimentation is needed to determine exposure time and concentration to elicit desired physiological effects in wound healing.[6]

As mentioned earlier, the toxicity of ozone has slowed the advancement of experimental data with human trials. Therefore, the purpose of this systematic review is to gather appropriate literature on ozone therapy, investigating the possibility of this treatment as a potentially effective medical procedure for the intervention of chronic wounds.

Key Messages

- wounds may become chronic when they do not progress past an inflammatory state
- ozone is a potential treatment for chronic wounds that is already applied in dentistry
- ozone treatment is thought to lead to mild oxidative stress and to have disinfectant properties, these features may help wounds move past the inflammatory state and aid in healing
- ozone therapy has not been widely adopted due to toxicity concerns
- meta-analysis found a significant improvement in wound closure with ozone therapy compared to control treatments, and no adverse effects linked directly to ozone therapy were reported in any study

2 | METHODS

2.1 | Inclusion criteria

2.1.1 | Types of studies

Randomised controlled trials (RCTs) were considered for inclusion.

2.1.2 | Types of participants

The selected studies included human subjects of any age with chronic wounds, including war wounds, burns, and non-healing diabetes, venous, or arterial ulcers.

2.1.3 | Types of interventions

Intervention group: Receiving treatment with an advanced wound care therapy of ozone in either gaseous, water, or oil form.

Control group: Participants subjected only to standard wound care or baseline values of intervention group before participants were subjected to the ozone intervention.

2.2 | Types of outcome measures

2.2.1 | Primary outcomes

Primary outcomes included the number of ulcers completely healed in the trial period, the measured change in wound size in trial period, presence or absence of biomarkers in favour of healing, and—for diabetic foot ulcers—the general appearance of the wound as summarised in the level of reported difference at end of the trial period, as assessed by Wagner's ulcer classification scale (Table 1).

2.2.2 | Secondary outcomes

Secondary outcomes included the complications of pain, toxicity, amputation, infection, and developed pathologies.

2.3 | Data collection

2.3.1 | Data sources and searches

MEDLINE and Google Scholar were searched for preferably randomised controlled trials published in any time frame (Figure 1). The searches were limited to English-language studies involving human subjects of all ages. Additional references were obtained from a search of the Cochrane Library, existing systematic reviews, and reference lists.

2.3.2 | Data collection and analysis

Abstracts were reviewed and identified from the literature search for relevance. The studies included were of human subjects with chronic wounds receiving treatment with an advanced wound care therapy of ozone in either gaseous, water, or oil form. Studies were included if they compared these therapies with standard wound care as well as with other advanced therapies and reported either percentage of wounds completely healed at study completion or the level of healing by either measured change or general appearance.

2.3.3 | Study selection

Results of all searches were screened for the following information: title, author, source; clinical trial; presence of chronic wound; patient characteristics; patient exclusion/inclusion criteria; balance of groups at baseline; allocation of concealment; sample size; *P*-value for ozone treatment compared with control; intervention details, dosage, exposure time; manner of ozone application; length of treatment; outcome measures; blinding of the patient and/or assessor; reasons behind patient dropouts; results; and detailed conclusion. Full-text copies of all studies meeting the inclusion criteria were obtained for detailed assessment.

2.4 | Study assessment

2.4.1 | Assessment for level of bias

Data extraction and validity assessment were conducted independently. Assessment of validity included the following criteria (Table 2):

TABLE 1 Wagner's ulcer classification scale

Wagner score	Description
Grade 0	No open ulceration, but with possible existence of bone deformation of hyperkeratosis
Grade 1	Superficial ulceration, but without penetration to deeper tissues
Grade 2	Deeper extension into tendons, bones, or joint capsule, which may be exposed
Grade 3	Presence of tendonitis, osteomyelitis, cellulitis, or deeper tissue abscess
Grade 4	Wet or dry gangrene of toe or dorsum of the foot, often with plantar infection
Grade 5	Extensive gangrene of the foot, with necrotic lesions and soft tissue infections indicating higher amputation

- Random sequence generation (selection bias), *"Was the allocation method randomly instigated?"* (high/low/unclear)
- Allocation concealment (selection bias), *"Was the treatment concealed from the patient?"* (high/low/unclear)
- Blinding of participants and researchers (performance bias), *"Were the researchers blinded to the treatment being administered"* (high/low/unclear)
- Incomplete outcome data (attrition bias), *"Was missing data adequately addressed?"* (high/low/unclear)
- Selective reporting (reporting bias), *"Are the reports of the study free of selective outcome data?"* (high/low/unclear)

Trials were judged overall has having high, low, or moderate risk bias on the following basis, with all "unclear bias" counted as high risk (Table 3):

- High risk: $\leq$ 1 low risk bias
- Low risk: $\leq$ 1 high risk bias
- Moderate risk: > 1 high risk bias AND >1 low risk bias

2.4.2 | Measures of treatment effect

Meta-analysis of the data was performed using the mean standard difference. Trials were analysed on a *P*-value score of ozone treatment vs control. Mean *P*-values and 95% confidence intervals were calculated using the web-based programme Practical Meta-Analysis Effect Size Calculator (www.campbellcollaboration.org/escalc/html/EffectSizeCalculator-Home.php).

2.4.3 | Dealing with missing data

Attempts were made to contact study authors where inclusion was unclear (due to difficulties accessing full-text article). Where such information was not obtained, extensive analysis of the abstract was conducted for a possibility of obtaining relevant data.

2.4.4 | Assessment of heterogeneity

Clinical heterogeneity was assessed by examining study characteristics in the included studies and calculating the common *P*-value effect.

2.4.5 | Data synthesis

The consistent, calculated *P*-value was used as a fixed-effects method for meta-analysis.

2.4.6 | Results presentation

Results for continuous data are presented as either mean difference (MD) or standardised mean difference (SMD). The SMD compares the significance of treatment across all studies despite differing treatments types in studies. The calculated SMD is the difference in mean outcome across all

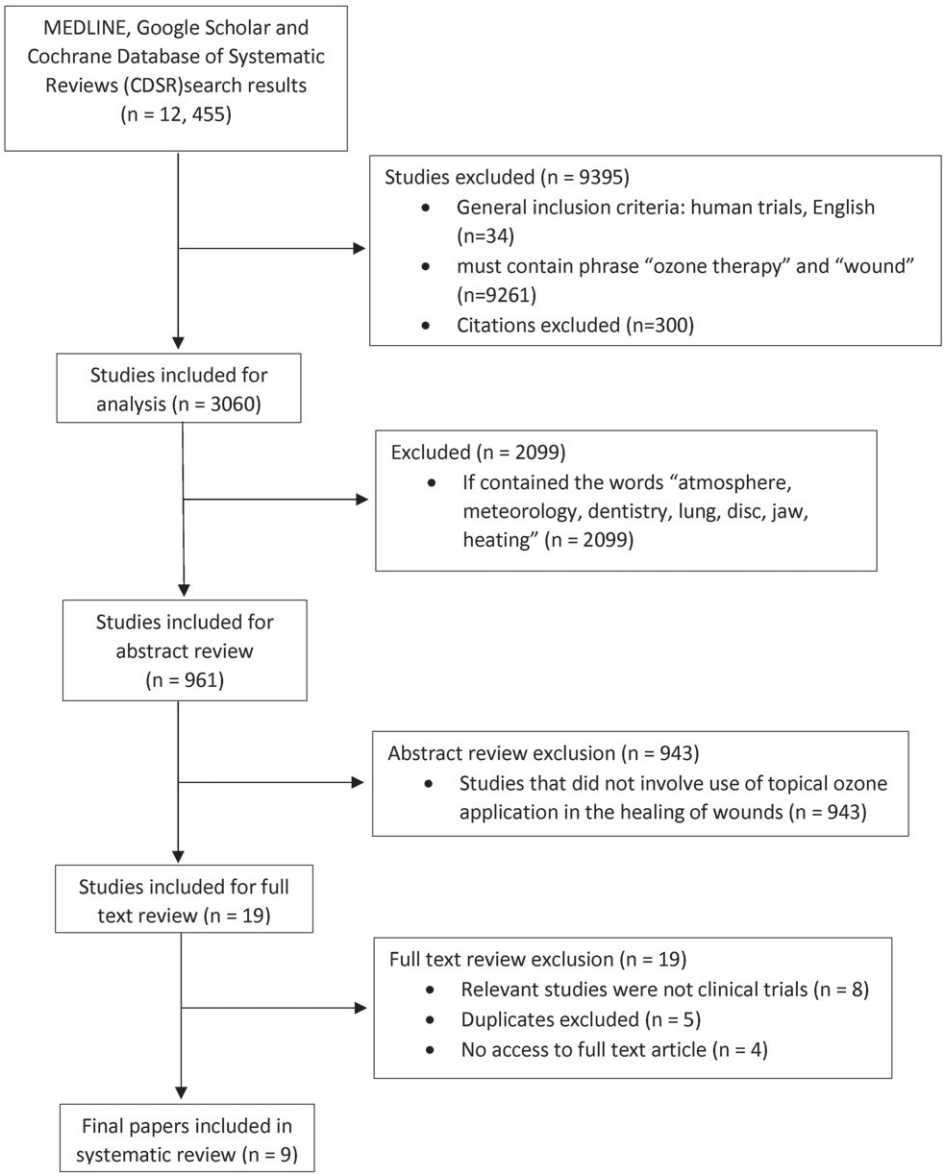


FIGURE 1 Flow diagram of the search strategy and study selection. A total of 12 455 studies were retrieved from Google Scholar, Medline databases, Cochrane Library, existing systematic reviews, and reference lists. After excluding irrelevant studies, duplicates, and studies where full texts could not be accessed, 9 studies were assessed eligible and included in this systematic review

groups divided by the standard deviation of outcome among participants.

3 | RESULTS

3.1 | Results of search and description of studies

A total of 12 455 titles and abstracts were retrieved from searches. Of these, 11 494 were excluded because the articles were not in English; were animal trials; were citations; did not specifically contain the phrase "ozone therapy"; or contained the words "atmosphere, meteorology, dentistry, lung, disc, jaw, or heating". The exclusion words were related to common applications of ozone not related to the studies discussed in the current review. After performing a detailed review of the remaining 961 articles, 943 studies were found to be unrelated to the use of ozone as a topical therapy for wounds. Of the remaining 19 studies, 9 were not clinical trials, 6 were duplicates, and 4 full-text articles could not be accessed (Figure 1).

Nine eligible trials with a combined total of 453 participants were identified. Trials subjected chronic wounds to ozone therapy either as an ozone-oxygen gas mixture or ozonated oil (Figure 1; Table 4). The chronic wounds included diabetic ulcers (3 studies;[27,28,31]), venous and arterial ulcers (4 studies;[13,26,29,30]), gunshot wounds (1 study;[6]), and chronic burns (1 study;[18] Table 4). All study subjects were treated with standard wound care for the chronic wounds present. Five studies compared ozone therapy with standard care or a placebo. In 3 studies, participants were subjected to ozone therapy with their prior baseline wound condition as the control (Table 4).

3.2 | Outcomes

3.2.1 | Primary outcomes

Meta-analysis of the 9 studies ($n = 453$ patients) revealed a significant ($P < .05$) improvement in wound closure

TABLE 2 Summary of the assessment of bias criteria used for selected studies

Type of bias	Judgement criteria
Random sequence generation (selection bias)	**Low risk:** investigators clearly outlined randomisation had occurred in 1 or more aspects of the study
"Was the allocation method randomly instigated?"	**High risk:** investigators note a non-randomised aspect of the generation of outcomes
	Unclear: insufficient information to judge a "*high*" or "*low*" bias assessment
Allocation concealment (selection bias)	**Low risk:** participants were blinded to the treatment they were being given
"Was the treatment concealed from the patient?"	**High risk:** participants had full awareness of what treatment they were exposed to
	Unclear: insufficient information to judge a "*high*" or "*low*" bias assessment
Blinding of participants and researchers (performance bias)	**Low risk:** researchers and participants were blinded to the treatment being given
	High risk: researchers had full awareness of what treatment they were administering
"Were the researchers blinded to the treatment being administered?"	**Unclear:** insufficient information to judge a "*high*" or "*low*" bias assessment
Incomplete outcome data (attrition bias)	**Low risk:** *Any 1 of the following:* The number and reasons for 'drop outs' were detailed. Mention of altered statistical analysis to account for incompletion of treatment. 'Drop outs' did not affect sample size. All outcome data were recorded. Patient characteristics that may impact outcomes were detailed
"Was missing data adequately addressed?"	**High risk:** *Any 1 of the following:* The number and reasons for 'drop outs' were not recorded. No statistical alteration was made due to incompletion of treatment. "Drop outs" significantly impacted sample size. Outcome data were missing. Patient characteristics that may impact outcomes were not detailed
	Unclear: insufficient information to judge a "*high*" or "*low*" bias assessment
Selective reporting (reporting bias)	**Low risk:** *Any 1 of the following:* The study protocol is detailed, and all pre-specified primary and secondary outcomes were addressed. The study protocol is not specified; however, all reports clearly indicate all studies addressed all expected outcomes
"Are the reports of the study free of selective outcome data?"	**High risk:** *Any 1 of the following:* The study protocol is not detailed. The protocol is detailed; however, 1 or more pre-specified primary and secondary outcomes of the study were not addressed. One or more outcomes were addressed with no pre-specification. Outcomes that occurred in the study that should have been noted were not
	Unclear: insufficient information to judge a "*high*" or "*low*" bias assessment

(wound healing and percent wound closure) with ozone therapy compared with the control. All studies demonstrated improvements in primary outcomes with ozone therapy (Figure 2). The difference in outcome favouring ozone therapy over standard care was very high (mean standard difference [MSD] was between 0.99 and 0.92) for 4 trials[6,18,28,29] and moderate to low (MSD was between 0.78 and 0.52) for 5 trials;[13,26,27,30,31] Figure 2).

All studies reported results with respect to the primary outcome of change in healing of chronic wounds at study conclusion (Table 5). Two studies reported a percentage amount of full wound closure; Wei et al[30] reported a 13.6% and Wainstien et al[31] reported a 37% superiority in the amount of full ulcer closures using ozone compared with the control. Marfella et al [26] demonstrated a 50% decrease in biochemical markers that prevent healing with ozone compared with 7% in the control group. Four studies focused on the amount of ulcer closure and general appearance of the wound[27–30]; these studies found a 24%,[27] 50%,[29] and 25%[13] difference in favour of the ozone treatment. Zang et al[28] found that ozone increased closure of ulcers by an average of 3.65 cm^2 compared with the control. One study[6] assessed closure of gunshot wounds and found 75% of patients experienced wound closure with ozone compared with 40% in the control group. Campanati et al[18] assessed the ozone's effects on burn patients and noted that the erythema reduced from a baseline score of 2.16 to 1.23.

3.2.2 | Secondary outcomes

No adverse effects linked directly to ozone therapy were reported in any study. No participants were reported to have dropped out of therapy as a direct cause of the intervention (drop outs were related to comorbidities, inability to follow up, deaths, or complications unrelated to the intervention[26,31]). In the study by Wainstein et al,[31] 2 of the 32 intervention participants dropped out due to their condition worsening; whether this was directly due to the ozone treatment is unclear. Marfella et al,[26] Borrelli et al,[29] and Campanati et al[18] reported a significant decrease in pain perception following the ozone intervention.

3.3 | Method of ozone delivery

Ozone may be delivered to the wound site as a gas or infused in oil, and the delivery method may influence the effectiveness of treatment. Five studies exposed wounds to the ozone treatment using an ozone-gas mixture within an airtight container and found significant ($P = .03$, $P = .01$, $P = .001$, $P = .01$, $P = .03$) improvement in would healing compared with controls.[6,15,27,28,31] One study bubbled patient's blood with an ozone-oxygen gas mixture before

TABLE 3 Assessment of risk of bias

Study	Type of bias	Judgement on risk of bias	Evidence and comments
Marfella et al[26]	Random sequence generation (selection bias)	Low risk	"Eligible patients were randomly allocated in a one to one manner"
	Allocation concealment (selection bias)	Low risk	Patients allocated to the placebo group were subjected to the same protocols as the treatment group; however, they were injected with a saline solution rather than ozonides
	Blinding of participants and researchers (performance bias)	Unclear[a]	There was not mention of blinding of the physician
	Incomplete outcome data (attrition bias)	Low risk	All patients completed the treatment
			The reason for patient exclusion was detailed
			Safety guidelines and adverse effects were noted
			Patient demographics and medical history was detailed
	Selective reporting (reporting bias)	Low risk	All pre-specified outcomes were reported
	Overall assessment of bias: Low risk		
Martínez-Sánchez et al[27]	Random sequence generation (selection bias)	Low risk	"Patients were randomised to two different groups of treatment"
	Allocation concealment (selection bias)	High risk	It was clear which groups were receiving the oxygen-ozone treatment
	Blinding of participants and researchers (performance bias)	High risk	Physicians were aware which treatment was delivered
	Incomplete outcome data (attrition bias)	Low risk	The reason for patient exclusion was detailed
			Patient demographics and medical history was detailed
			Unclear of patient drop outs
	Selective reporting (reporting bias)	Low risk	All pre-specified outcomes were reported
	Overall assessment of bias: Low risk		
Zhang et al[28]	Random sequence generation (selection bias)	Low risk	Patients were randomly allocated to 1 of 2 groups
	Allocation concealment (selection bias)	High risk	It was highly obvious which groups were receiving the oxygen-ozone treatment
	Blinding of participants and researchers (performance bias)	High risk	The physicians only applied the special bag to patients receiving the ozone treatment
	Incomplete outcome data (attrition bias)	Low risk	All patients finished the treatment
			The exclusion criteria for patients were noted
			Patient demographics and medical records were obtained and noted
	Selective reporting (reporting bias)	Low risk	All pre-specified outcomes were reported
	Overall assessment of bias: Moderate risk		
Turcić et al[6]	Random sequence generation (selection bias)	low risk	Patients with specific criteria were randomly assigned to control or ozone group
	Allocation concealment (selection bias)	High risk	Patients were aware which wounds would be exposed to the ozone treatment
	Blinding of participants and researchers (performance bias)	High risk	Physicians were aware which wounds were treated with ozone
	Incomplete outcome data (attrition bias)	High risk	All patients finished the treatment. However, no other information on excluded data could be gathered
	Selective reporting (reporting bias)	Low risk	All pre-specified outcomes were reported
	Overall assessment of bias: Moderate risk		
Borrelli et al[29]	Random sequence generation (selection bias)	High risk	Patients were selected based on specific criteria and were divided into groups depending on that criteria
	Allocation concealment (selection bias)	High risk	Patients signed an informed consent form and were aware what treatment they were receiving
	Blinding of participants and researchers (performance bias)	High risk	Physicians were aware which treatment they were administering
	Incomplete outcome data (attrition bias)	Low risk	All patients completed the treatment
			Demographic and medical characteristics were noted
	Selective reporting (reporting bias)	Low risk	All primary and secondary outcomes were noted and addressed
	Overall assessment of bias: Moderate risk		
Wei et al[30]	Random sequence generation (selection bias)	Low risk	

(*Continues*)

TABLE 3 (Continued)

Study	Type of bias	Judgement on risk of bias	Evidence and comments
			Patients were randomly assigned to either a control or treatment group
	Allocation concealment (selection bias)	High risk	Patients signed an informed consent form and were aware what treatment they were receiving
	Blinding of participants and researchers (performance bias)	High risk	Physicians were aware which treatment they were administering
	Incomplete outcome data (attrition bias)	Low risk	All patients completed the treatment with no severe complications
			All minor complications were documented
	Selective reporting (reporting bias)	Low risk	All primary and secondary outcomes were noted and addressed
	Overall assessment of bias: Moderate risk		
Campanati et al[18]	Random sequence generation (selection bias)	High risk	The study was non-randomised as both treatments were applied to each patient
	Allocation concealment (selection bias)	Low risk	"The patients were blinded on which part of the burn they applied respectively ozonides or hyaluronic acid"
	Blinding of participants and researchers (performance bias)	High risk	The physician knew which part of the wound they applied the ozonides and hyaluronic acid
	Incomplete outcome data (attrition bias)	low risk	It was noted that patients were excluded if they suffered from various specific disorders; however, the number of patients excluded was unclear
	Selective reporting (reporting bias)	Low risk	An external physician was used to conduct quantitative analysis
			Questions used for patient's self-assessment of the treatment were noted within the study, and they remained vague and open ended
	Overall assessment of bias: Moderate risk		
Solovăstru et al[13]	Random sequence generation (selection bias)	Low risk	Patients were randomly assigned to 1 of 2 groups
	Allocation concealment (selection bias)	High risk	Patients were aware they were receiving either the ozonated oil and α-bisabolol or the control cream
	Blinding of participants and researchers (performance bias)	High risk	Physicians administering the treatment were aware which patients were control selected for the ozone treatment
	Incomplete outcome data (attrition bias)	Unclear	
	Selective reporting (reporting bias)	Low risk	All pre-specified outcomes were reported
	Overall assessment of bias: Moderate risk		
Wainstein et al[31]	Random sequence generation (selection bias)	Low risk	Patients were randomly assigned to 1 of 2 groups
	Allocation concealment (selection bias)	Low risk	Patients allocated to the control group received the same protocols as the ozone group; however, they were exposed to room air as opposed to the oxygen-ozone solution
	Blinding of participants and researchers (performance bias)	Low risk	As a double blind study, the physician was unaware if patients were exposed to ozone
	Incomplete outcome data (attrition bias)	Low risk	The number and reason for patient exclusion and withdrawal were detailed
			Patients who missed more than 1 session or did not complete the treatment were analysed separately as non-completer and completer groups, respectively
	Selective reporting (reporting bias)	Low risk	All primary and secondary outcomes were noted and addressed

Determination of overall assessment of bias: High risk: ≤ 1 low risk bias. Low risk: ≤ 1 high risk bias. Moderate risk: > 1 high risk bias AND >1 low risk bias.

[a] All "unclear bias" was counted as "high risk" for that section of bias. Overall classification of the study was determined by the following: High risk: ≤ 1 low risk bias. Low risk: ≤ 1 high risk bias. Moderate risk: > 1 high risk bias AND >1 low risk bias. The graph demonstrates that 2 of the studies selected were determined as low risk; 6 were determined as moderate risk, with no studies labelled as having a high risk of bias.

reinfusion and found a significant ($P = .001$) decrease in inflammatory biomarkers (50%) compared with the control (10%).[26] One study blew an oxygen-ozone mixture directly into the cavity using a catheter and found an 85% wound closure rate compared with the 71% in the control group ($P = .05$).[30] Of the 2 studies that used ozonised oil as a treatment method,[13,18] Campanati et al[18] demonstrated a significant ($P = .003$) improvement in wound healing compared with the control, and Solovăstru. 2015[13] 25% wound closures compared with the control, and the results were also statistically significant ($P = .05$) (Tables 4 and 5).

TABLE 4 Summary of baseline characteristics of studies

Characteristic	Marfella et al[26]	Martínez-Sánchez et al[27]	Zhang et al[28]	Turcić et al[6]	Borrelli et al[29]	Wei et al,[30]	Campanati et al[18]	Solovăstru et al[13]	Wainstein., et al[31]
Ozone treatment	Oxygen/ozone gas mixture by intra-gluteal injection	Rectal insufflation, non-invasive oxygen-ozone treatments in a specialised bag	Non-invasive oxygen-ozone treatments in a specialised bag	Non-invasive oxygen-ozone treatments in a specialised bag	Topical exposure of oxygen-ozone mixture	Insertion of catheter and ozone was blown into cavity	Ozonated oil	Ozonated oil and α-bisabolol	Non-invasive ozone-oxygen in a hyperbaric chamber
Number of subjects randomly assigned	Control: 74 Treatment: 77	Control: 49 Ozone: 51	Control: 25 Treatment: 25	35[a]	Control: 16 Treatment: 8	Control: 21 Treatment: 26	30	29	Control: 16 Treatment: 18
Age (year) [median ± SD]	Control: 69 ± 7 Treatment:70 ± 5	20 to 80 years	Control: 61 ± 11 Treatment: 60 ± 12	25 ± 4	Control: 64.5 ± 14.5 Treatment: 64.4 ± 16.1	Control: 51.5 Treatment: 48.4	52.5 ± 14.3	18 to 30 years	Control: 62.6 ± 9.5 Treatment: 62.6 ± 10.2
Gender [male, *n* (%)]	Control: 52 (70%) Treatment: 54 (70%)	Control: 30 (61%) Treatment: 26 (50%)	Control: 12 (48%) Treatment: 14 (56%)	35 (100%)	Control: 5 (62.5%) Treatment: 4 (50%)	Control: 17 (81%) Treatment: 15 (58%)	11 (36.6%)	Unknown	Control: 19 (66%) Treatment: 19 (54%)
Condition	Poor perfusion ulceration	Diabetes patients with Wagner classification stage 2 or 3 ulcer or stage 4 ulcer after debridement	Diabetes patients with a Wagner classification stage 2 or 3 ulcer or a stage 4 ulcer	Gunshot wounds	Ulceration from arterial ischaemia	Chronic refractory sinus and ulcer	Skin burns in the phase of reepithelisation	chronic venous leg ulcers	Diabetes patients with a Wagner classification stage 2 or 3 ulcer or a stage 4 ulcer
Treatment duration	22 weeks	20 days	20 days	10 days	2 days	25 days	12 weeks	30 days	12 weeks
Frequency of treatment (total)	1/week (22)	1/day (20)	30 min/day (20)	Ozone was performed every other day (5)	1/day (2)	1/day for 20 min (25)	1/day (63)	1/day (30)	(1) 4/week - 4 weeks (2) 2/week for 12 weeks (32)
Concentration of treatment	15.35 μg/mL ozone	Rectal unsufflation: 50 mg/L Topical: 60 mg/L	52 μg/mL ozone	30-50 μg/mL ozone	60 μg/mL ozone	40 μg/mL ozone	Unknown	Unknown	(1) 80 μg/mL ozone (2) 40 μg/mL ozone[b]

[a] Controls were baseline values of treatment outcomes.

[b] Treatment was divided into 2 parts, initially with 80 μg/mL ozone treatment for 4 weeks or until wound was covered by 50% granulation tissue and then decreased to 40 μg/mL ozone.

Study	Number of subjects	p-value of t-test	Standardised mean difference (95% CI)	Varience
Marfella et al. (2010)	151	0.001	0.5465 (0.2215, 0.8714)	0.027489
Martínez-Sánchez et al. (2005)	100	0.03	0.5702 (0.0576, 1.0827)	0.068397
Zhang et al. (2014)	50	0.001	0.9914 (0.404, 1.5788)	0.089828
Turcić et al. (1995)	70	0.01	0.924 (0.227, 1.6211)	0.060009
E Borrelli et al, (2008)	32	0.01	0.9723 (0.2395, 1.705)	0.13977
Wei et al (2010)	47	0.05	0.5909 (0.0036, 1.1782)	0.089795
Campanati et al., (2013)	60	0.0003	0.9933 (0.4569, 1.5297)	0.074889
Solovăstru et al. (2015)	58	0.05	0.5261 (0.0025, 1.0496)	0.071351
Wainstein et al. (2011)	34	0.03	0.7804 (0.0819, 1.4789)	0.127011

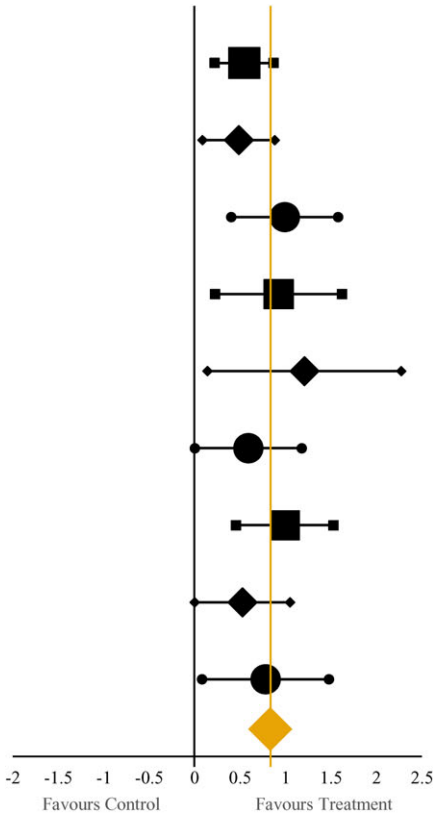


FIGURE 2 Meta-analysis of studies reporting on ozone treatment for chronic wounds. The outcomes in terms of wound healing and percent closure shift in favour of ozone treatment (*P*-value <.05). Dotted line shows mean

3.4 | Study participants

The mean age of participants in 7 of the 9 trials varied between 50 and 70 years, with the exclusion of Turcić et al,[6] and Solovastru et al,[13] where the mean age of participants ranged between 18 and 30 years and over 18 years, respectively. Five of the trials were conducted in Italy,[13,18,26,27,29] 2 in China,[28,30] 1 in Israel,[31] and 1 in Croatia.[6] An inclusion criterion for 5 trials was that the individuals presented with severe ulcerations from poor perfusion.[13,26,27,29,30] In 2 of the trials, patients were classified with a Wagner stage 2, 3, or 4 diabetic foot ulcer (Tables 1 and 4).[28,31] In 1 trial, participants presented with severe gunshot wounds located on the upper or lower limb.[6] One trial investigated the treatment on severe skin burns in the phase of reepithelisation.[18]

3.5 | Quality of evidence and data synthesis

The level of bias in 7 of the 9 studies was moderate, with bias in 2 or 3 domains (Figure 3, Table 3). Marfella et al[26] and Wainstein et al[31] demonstrated a low risk of bias, with bias in ≤1 domain. For most of the trials, a moderate bias score was received due to an absence of blinding and allocation concealment bias (Figure 3, Table 3). The Turcić et al 1995[6] study was classified as moderate risk of bias because the secondary outcome data could not be determined.

4 | DISCUSSION

The purpose of this review was to determine the efficacy of ozone as an advanced therapy for the treatment of chronic wounds. Nine trials were included with a combined total of 453 participants suffering from gunshot wounds, severe burns, and ulcers (diabetic, ischaemic or venous). Meta-analysis found evidence in favour of ozone treatment as all trials showed a significant improvement in healing when compared with the control. Therefore, ozone shows potential as an effective therapy in chronic wound care.

The 9 studies included within this review were mostly of high standard and low bias. When analysed together, the studies covered a broad spectrum of factors, including varying ulcer size and severity, differing ulcer types, differing chronic wound types, a large age range, and varying application methods and dosages of ozone. Most of the studies gave a detailed description of participant demographics and health status, and all studies mentioned reasons for non-adherence to the trial.

TABLE 5 Summary of wound-healing effect of ozone treatments

Study (year)	Ozone treatment method (*n*)	Control (*n*)	Wound-healing effect		*P*-value
Oxygen-ozone					
Marfella et al[26]	Blood exposed to oxygen/ozone gas mixture by intra-gluteal injection (74)	Non-specific immunomodulation therapy (77)	Ozone	Immunomodulation therapy	Ozone vs immunomodulation therapy
			< TNF-α[a] levels from 225.5 ± 28.2 to 114.8 ± 23.1 ng/L	<TNF-α levels 224.2 ± 29.2 to 207.9 ± 24.1 ng/L	$P < .001$
Martínez-Sánchez et al[27]	Rectal insufflation and non-invasive oxygen-ozone treatments in a specialised bag (51)	Topical application of antibiotic therapy (49)	Ozone	Placebo	Ozone vs placebo
			Area reduction percentage (% ± SD): 74.58 ± 0.35	Area reduction percentage (% ± SD): 50.30 ± 0.17	$P < .017$
Zhang et al[28]	Non-invasive oxygen-ozone in a special bag (25)	Standard treatment of debridement (25)	Ozone	Control	Ozone vs Control
			Change in wound size (cm^2 mean ± SD): 6.84 ± 0.62	Change in wound size (cm^2 mean ± SD): 3.19 ± 0.65	$P < .001$
Turcić, Hancevic, Antoljak, Zic, and Alfirevic (1995)	Ozone-oxygen mixture on lower limb wounds (35)	Conventional manners of 10% NaCl solution on upper limb wounds (35)	Ozone	Control	Ozone vs Control
			75% experienced wound closure	40% experienced wound closure	$P < .01$
Borrelli, et al[29]	Ozone-oxygen mixture (16)	Standard treatment (16)	Ozone	Control	Ozone vs Control
			80% experienced wound closure	30% experienced wound closure	$P < .01$
Wei et al[30]	Ozone-oxygen mixture (26)	Standard treatment (21)	Ozone	Control	Ozone vs Control
			85% experienced wound closure	71.41%	$P < .05$*
Wainstein, et al[31]	Ozone-oxygen mixture, applied in a sealed chamber (16)	Inactive ozone exposure (18)	Ozone	Placebo	Ozone vs placebo
			81% experienced full wound closure	44% experiences full wound closure	$P = .03$
Ozonated oil					
Campanati et al[18]	Occlusive application of ozonated oil (30)	Baseline (30)	Ozone	Baseline (T_0)	Ozone vs baseline
			(T_{12} – Oz)[b] erythema change 1.23 ± 0.85	Erythema 2.16 ± 0.91	*P*-value = .0003
Solovăstru et al[13]	Spray formulation containing ozonated oil and α-bisabolol (29)	Control cream of vitamin A, vitamin E, talc, and zinc oxide (29)	Ozone	Control cream	Ozone vs Control cream
			25% experienced full wound closure	0% experienced full wound closure	$P < .05$*

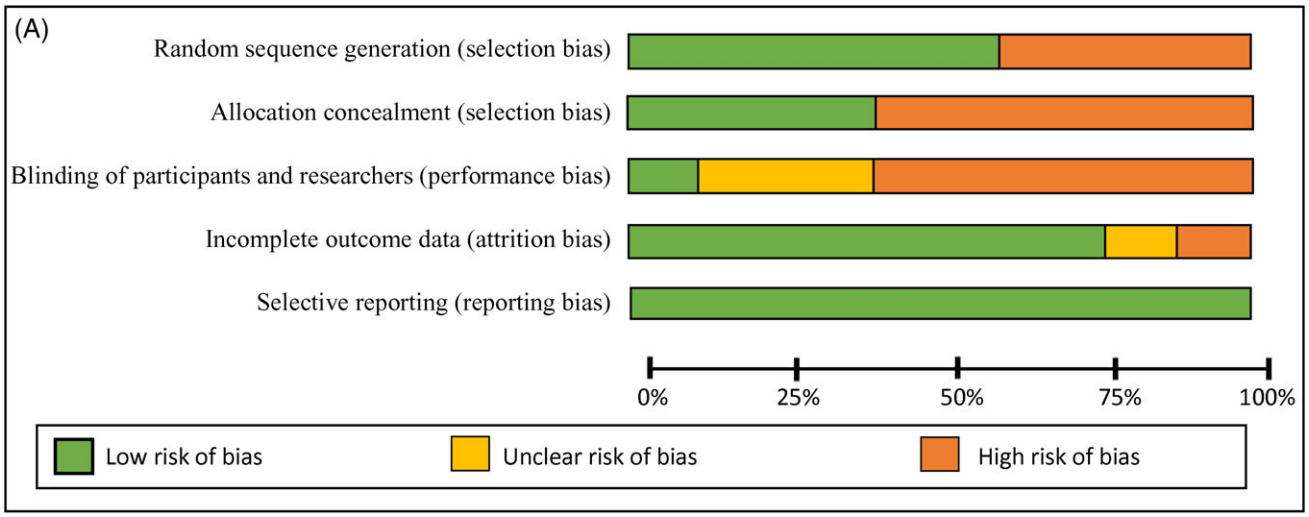
[a] TNF-α, tumour necrosis factor, a mediator of inflammation that directly inhibits wound healing.

[b] T_{12}, 12 weeks of treatment from baseline.

**P*-value <.05 is significant.

This review summarises the results of trials of ozone therapy in the treatment of chronic wounds. However, due to limited randomised clinical trials, the overall validity of conclusions is reduced. Only 4 randomised clinical trials were included in this review ($n = 276$ participants), assessing oxygen-ozone treatment within a sealed container.[6,27,28,31] Of the 4 randomised clinical trials, the duration and concentration of the ozone dose was different. Furthermore, these studies had some bias in the concealment of treatment. The current application methods of ozone can be an intricate procedure and typically require a trained professional, leading to the observed bias in the allocation and blinding of treatment. In addition, identifying patients with chronic wounds who meet inclusion criteria requires careful selection, with random sequence bias witnessed in studies using baseline as a control. Further, the Wagner classification scale chosen by the studies to categorise diabetic foot ulcers has been shown to be less reliable then other classification methods.[32] For the studies featuring different chronic wounds,[6,13,20,26,29,30] no classification scales for wound healing/severity were used by the authors. Such treatment differences and bias across the studies limits the conclusions that can be drawn.

This review included studies using the many application methods of ozone, including ozone-oxygen mixture exposure via a sealed container,[6,27–29,31] ozone-oxygen mixture bubbled through blood before being re-infused[26]; ozone-oxygen directly onto the wound via a catheter,[30] and ozonated oil.[13,18] Research has yet to be conducted to directly compare the effects of ozone-oxygen and ozonated oils. Such research would enable the most effective ozone treatment application method to be determined. Thus, the



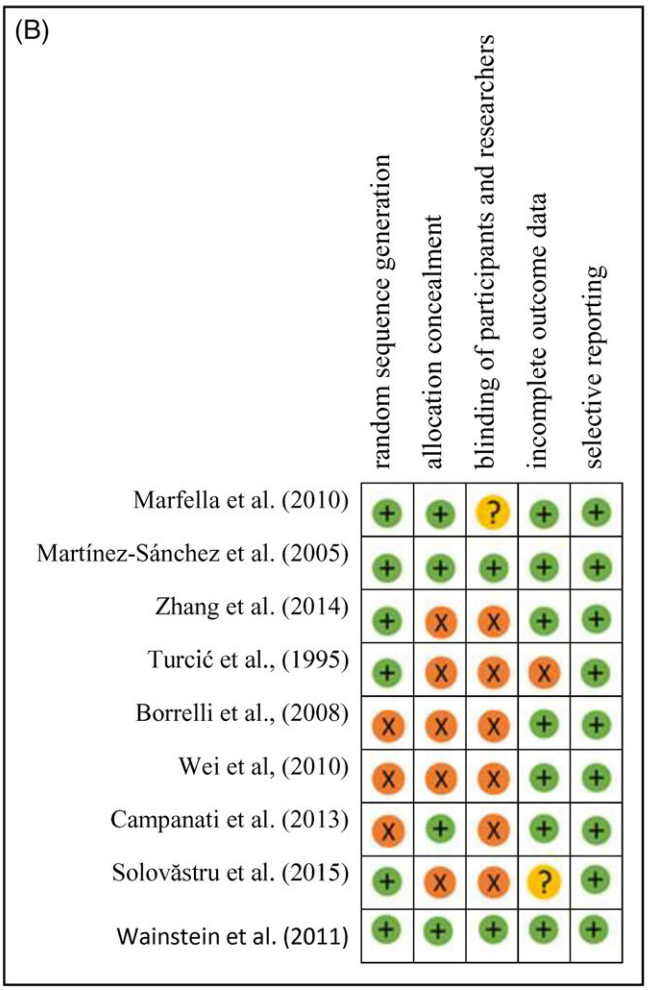


FIGURE 3 Risk of bias in studies. (A) Risk of bias item presented as percentages across all included trials. Visual representation of the measure of bias in each bias category across all studies. Green, low risk of bias; Yellow, moderate risk of bias; Red, high risk of bias. There is low risk of attrition and reporting bias across all studies but higher levels of bias with regards to selection and performance bias. (B) Detailed visual bias assessment of each study. Green "+", low level of bias; Yellow "?", moderate levels of bias; Red "X", high level bias. Of the 9 studies, 6 did not effectively blind their participants or researchers; however, 5 of the studies were of low bias in the other areas

specific methods and guidelines for ozone application are yet to be determined, and the guidelines and recommendations for ozone therapy have not yet been consolidated,[8] potenetially leading to the highly variable concentrations and dosage amounts in the studies collected for this review.

Of the 9 clinical trials within this review, 8 focused on wound healing as their primary outcome.[6,13,18,27–31] These studies also gave detailed descriptions of relevant secondary outcomes and concluded that the application of ozone at low concentrations had no adverse effects under the

conditions of the trials. However, due to ozone's known toxicity, further research must be conducted to determine the specific application methods of this treatment as well as to address additional outcomes of importance, including any possible long-term side effects that have not been previously reported. Such investigations will enable the most efficacious doses and application methods to be developed to maintain the delicate balance of ozone's potentially damaging oxidising effect with the treatment's medical benefits.

5 | CONCLUSIONS

Currently, there is no conclusive evidence of ozone therapy as a superior treatment for chronic wounds compared with standard treatments. However, results consistently favour the application of ozone as a treatment for chronic wounds, suggesting potential for mainstream clinical practice. The studies reviewed here include a broad range of participant ages and demographics, chronic wounds, and ozone application methods. This heterogeneity and the small number of current investigations limit the conclusions that can be drawn. Therefore, more research should be performed to consolidate the findings present and ensure consistency before clinical practice can be considered. Future research should focus on the precise dosage and timing of application and the specific procedure of application and should explore innovative approaches to application. Furthermore, it is vital that future research continue to evaluate the biological effects of ozone therapy and undertake more clinical double-blind trials with a long-term follow up to evaluate any long-term toxicity. The results of this meta-analysis suggest that there is good evidence to support ozone therapy as a potentially effective medical procedure for the treatment of chronic wounds, which requires further investigation.

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