MedNEXT
Journal of Medical an Health Sciences

REVIEW ARTICLE

DOI: 10.54448/mdnt25S207

Positive clinical effect of ozone therapy in dentistry through randomized clinical studies and meta-analysis: a systematic review

Cristina Ponsoni[1,2*], **Maria Júlia Gonçalves**[1,2], **Andreia Borges Scriboni** [1,2]

[1] UNORTE - University Center of Northern São Paulo, Dentistry Department, São José do Rio Preto, São Paulo, Brazil.
[2] UNIPOS - Post Graduate and Continuing Education, Dentistry Department, São José do Rio Preto, São Paulo, Brazil.

*Corresponding author: Cristina Ponsoni.
Unorte/Unipos. Graduate and Postgraduate education,
Dentistry department, São José do Rio Preto, São Paulo, Brazil.
E-mail: crisponsoni83@gmail.com
DOI: https://doi.org/10.54448/mdnt25S207
Received: 12-20-2024; Revised: 03-04-2025; Accepted: 03-18-2025; Published: 03-21-2025; MedNEXT-id: e25S207

Editor: Dr. Hae Shin Chung MD, Ph.D.

Abstract

Introduction: In dental treatment and oral health, ozone in low concentrations has been used successfully due to its oxidizing and antimicrobial properties. It also has anti-inflammatory and immunological action. **Objective:** A systematic review was developed to demonstrate the positive clinical effect of ozone therapy in dentistry through randomized clinical studies and meta-analysis. **Methods:** The PRISMA Platform systematic review rules were followed. The search was conducted from November 2024 to January 2025 in the Scopus, PubMed, Science Direct, Scielo, and Google Scholar databases. The quality of the studies was based on the GRADE instrument and the risk of bias was analyzed according to the Cochrane instrument. **Results and Conclusion:** 111 articles were found, 38 articles were evaluated in full and 13 were included and developed in the present systematic review study. Considering the Cochrane tool for risk of bias, the overall assessment resulted in 23 studies with a high risk of bias and 30 studies that did not meet GRADE and AMSTAR-2. Most studies did not show homogeneity in their results, with X^2=75.8%>50%. It was concluded that ozone therapy is an alternative to accelerate healing and reduce pain in traumatic and autoimmune ulcers. Ozone offers a painless alternative to conventional treatment for dental caries and has been shown to arrest primary root caries, primary pit, and fissure caries, and clinically reverse the lesion. Its antimicrobial action on endodontic microbiota is quite promising. Ozone gel is suggested as an adjunctive therapy in diabetic patients to improve periodontal health. Oxygen-ozone therapy has been shown to enhance post-extraction healing in patients at risk for MRONJ. As for reducing the microbial load for patients undergoing root canal treatment, ozone therapy has inferior results when compared to conventional chemomechanical techniques using NaOCl.

Keywords: Oral treatment. Ozone therapy. Oral health. Anti-inflammatory. Immunity. Painless.

Introduction

In dental treatment and oral health, ozone in low concentrations has been successfully used due to its oxidizing and antimicrobial properties. It also has antiinflammatory and immunological action. In addition, ozone is effective in containing the formation of resistant microbial biofilms in the root canal. The antimicrobial action is due to ozone-induced oxidation, which causes direct and indirect damage to the structures and metabolism of microbial cells [1].

It is observed that ozone is also involved in pharmacological immunomodulation, as it induces mild oxidative stress, which triggers antioxidant responses through the activation of specific molecular pathways, activating anti-inflammatory mechanisms. In addition, topical application of ozone has been reported to improve the rheological properties of blood, thus improving peripheral oxygen perfusion and general metabolism [2,3].

It is also noted that the recommended concentration for a mixture of oxygen and ozone for use in humans is between 5-50 µg of ozone/1 mL of oxygen. According to the guidelines and recommendations of the World Federation for Ozone Therapy, an ozone concentration of 5-10 µg/mL is recommended for the

application of ozonated products by intralesional injection or irrigation, to obtain a therapeutic effect without the risk of toxicity [3].

Despite this, some potential adverse effects may occur and should be considered, mainly toxicity by inhalation, causing headaches, vomiting, and irritation of the upper respiratory tract. In addition, certain forms of ozone therapy, that is, those that induce systemic effects, are contraindicated in myocardial infarction, hyperthyroidism, acute alcohol intoxication, severe anemia, thrombocytopenia, active hemorrhage, and pregnancy [4-7].

In this scenario, ozone therapy is indicated in implant dentistry, oral surgery, periodontology, oral medicine, and dental caries treatment. Ozone applications have produced promising results in the treatment of inflammatory and immune-mediated conditions of oral soft tissues, such as oral lichen planus and aphthous stomatitis [1-3]. In addition, ozone has been applied in the context of postoperative wounds and complications such as alveolar osteitis, reducing pain and healing time. In addition, ozone has contributed to the treatment of oral ulcerative conditions and surgical wounds. Furthermore, ozone can cause a remineralizing effect on dental hard tissues to treat demineralization and dental caries [8-13].

Also, ozone therapy is being tested as an alternative or co-acting agent to NaOCl. However, some authors have shown that ozone therapy has similar results compared to NaOCl in reducing various species of bacteria [14-16], while others have reported less efficacy [17]. The action of ozone, directly and indirectly, modulates the relationship of the patient's immune system, thus improving the body's response to the etiological agent. However, it is still necessary to define with scientific evidence the ability of the ozone molecule to stimulate biological effects, encouraging tissue repair, healing, and return of the tooth to its natural function. To all these attributions, it is understood that ozone thus has great potential to be included in endodontic therapy, as it requires and incorporates the two requirements necessary for any substance for endodontic use, such as antimicrobial action par excellence and biocompatibility [6,7].

Therefore, the present study developed a systematic review to demonstrate, through randomized clinical studies and meta-analysis, the positive clinical effect of ozone therapy in dentistry.

Methods

Study Design

This study followed the international systematic review model, following the PRISMA (preferred reporting items for systematic reviews and meta-analysis) rules. Available at: http://www.prisma-statement.org/?AspxAutoDetectCookieSupport=1. Accessed on: 02/10/2025. The AMSTAR 2 (Assessing the methodological quality of systematic reviews) methodological quality standards were also followed. Available at: https://amstar.ca/. Accessed on: 02/10/2025.

Search Strategy and Search Sources

The literature search process was carried out from November 2024 to January 2025 and developed based on Web of Science, Scopus, PubMed, Lilacs, Ebsco, Scielo, and Google Scholar, covering scientific articles from various periods to the present day. The following descriptors were used in health sciences (DeCS/MeSH): *Oral treatment. Ozone therapy. Oral health. Anti-inflammatory. Immunity. Painless*, and the Boolean "and" was used between the MeSH terms and "or" between the historical findings.

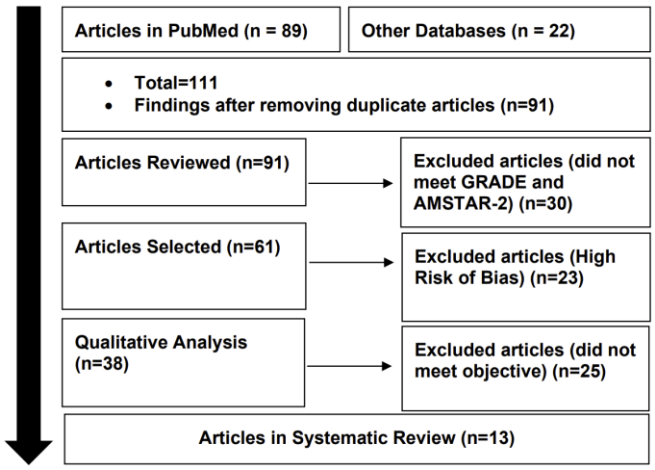
Study Quality and Risk of Bias

Quality was classified as high, moderate, low, or very low regarding the risk of bias, clarity of comparisons, precision, and consistency of analyses. The most evident emphasis was on systematic review articles or meta-analyses of randomized clinical trials, followed by randomized clinical trials. Low quality of evidence was attributed to case reports, editorials, and brief communications, according to the GRADE instrument. The risk of bias was analyzed according to the Cochrane instrument by analyzing the Funnel Plot graph (Sample size versus Effect size), using Cohen's test (d).

Summary of Findings

A total of 111 articles were found and submitted to eligibility analysis, with 13 final studies selected to compose the results of this systematic review. The listed studies were of medium to high quality (Figure 1), considering the level of scientific evidence of studies such as meta-analysis, consensus, randomized clinical, prospective, and observational. Biases did not compromise the scientific basis of the studies. According to the GRADE instrument, most studies presented homogeneity in their results, with X^2=75.8%>50%. Considering the Cochrane tool for risk of bias, the overall assessment resulted in 23 studies with a high risk of bias and 30 studies that did not meet GRADE and AMSTAR-2.

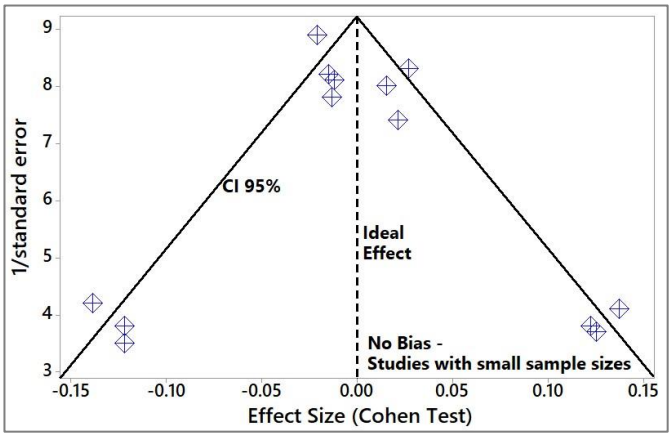
Figure 1. Flowchart showing the article selection process.



Source: Own Authorship.

Figure 2 presents the results of the risk of bias of the studies using the Funnel Plot, showing the calculation of the Effect Size (Magnitude of the difference) using Cohen's Test (d). Precision (sample size) was determined indirectly by the inverse of the standard error (1/Standard Error). This graph had a symmetrical behavior, not suggesting a significant risk of bias, both among studies with small sample sizes (lower precision) that are shown at the base of the graph and in studies with large sample sizes that are presented at the top.

Figure 2. The symmetrical funnel plot suggests no risk of bias among the studies with small sample sizes that are shown at the bottom of the graph. High confidence and high recommendation studies are shown above the graph (n=13 studies).



Source: Own Authorship.

Major Considerations and Clinical Results

The authors Maglia et al. (2024) [18], through a meta-analysis, evaluated the efficacy of ozone therapy in the healing of oral ulcers when compared to placebo or active treatments through 12 studies. It was shown that ozone therapy helps to reduce the size of traumatic and autoimmune ulcers (RR=-0.44; I^2 = 0%) compared to placebo. Regarding pain reduction, ozone was superior to placebo (RR=1.29; I^2 = 0%), and equivalent to topical corticosteroids and laser photobiomodulation (RR=0.26; p=0.34).

A study carried out by the authors Hashim et al. (2025) [19] showed the use of ozone therapy in periodontal disease, including selective antimicrobial action against biofilm-associated pathogens, immunomodulatory effects on host cells, and stimulation of tissue repair. Ozone therapy disrupts microbial biofilms, improves immune cell function, and promotes healing by activating the Nuclear Factor Erythroid 2-related Factor 2 (Nrf2) and Mitogen-Activated Protein Kinase (MAPK) signaling pathways that regulate oxidative stress, inflammation, and apoptosis. Advances in periodontal regenerative techniques, combined with the antimicrobial and wound-healing properties of ozone, have demonstrated significant clinical benefits.

In addition, the authors Barahim et al. (2024) [20] conducted a randomized controlled clinical trial to evaluate the clinical and radiographic outcomes of the effect of subgingival application of ozonated gel as an adjunct to scaling and root planing (SRP) in diabetic patients with stage III periodontitis. A total of 24 patients with type II diabetes mellitus (T2DM) were randomized into two groups, with 12 patients in each group. Group I served as the intervention group, receiving SRP and ozone gel application (SRP + Ozone), while Group II served as the control group, receiving SRP alone. The results revealed statistically significant intragroup differences between the two groups ($p < 0.05$). In contrast, intergroup differences revealed no statistically significant differences between the various time intervals ($p > 0.05$). The reduction in PD in the SRP + Ozone group at 3 months was statistically significant ($p = 0.04$). The SRP + Ozone group showed significant radiographic improvement compared to the SRP group. The Visual Analog Scale (VAS) also demonstrated statistically significant differences between the two groups. Glycated hemoglobin (HbA1c) decreased significantly after 6 months, with no significant differences between the groups ($p>0.05$).

Another randomized clinical trial analyzed the efficacy of ozonated gels compared with conventional chlorhexidine gel in the home treatment of periodontal patients. A total of 30 patients with bilateral periodontal disease (severity I, complexity II) were enrolled. After non-surgical mechanical periodontal debridement, the teeth were randomly divided into two groups: the teeth in the Control group were treated with a chlorhexidine-based gel to aid oral hygiene maneuvers for 2 weeks after the first visit, while the teeth in the Test group were treated in the same way with ozone-based gels. After the initial evaluation, follow-up included evaluations at 1, 2, and 6 months. Non-surgical

mechanical periodontal debridement with adjunctive use of ozone was considered effective in periodontal treatment. Ozone can be suggested as an alternative to chlorhexidine [21].

Also, the authors Di Fede et al. (2024) [22] evaluated the efficacy and safety of an oxygen-ozone gas mixture as an adjunct to standard tooth extraction to reduce the risk of developing drug-related osteonecrosis of the jaw (MRONJ) through a randomized controlled clinical trial. A total of 117 patients at risk of MRONJ were included. The oxygen-ozone therapy group exhibited significant improvement in post-extraction wound healing during the inflammatory and proliferative phases, as indicated by IPR scores at 3-5 days and 14 days, respectively.

Through the evaluation of selected studies, it was found that ozone was first suggested for root canal treatment because of its reported high antimicrobial action [23]. A significant decrease in oral cell cytotoxicity was observed with ozone gas compared to 2.25% NaOCl and 2% chlorhexidine gluconate [24,25]. Furthermore, aqueous ozone (up to 20 mg mL-1) was not toxic to oral cells [26].

A systematic review study revealed that the antimicrobial effect of ozone is strongly associated with the application protocol used, such as dose, time, and correlation with the use of complementary sources of disinfection. Also, ozone has different antimicrobial effects according to groups of bacteria (Gram-positive and Gram-negative). Since the structure of Gram-negative bacteria contains lipopolysaccharides (LPS) and phospholipids in the membrane, this group appears more susceptible to ozone [27].

A randomized trial analyzing the efficacy of ozone or NaOCl/Chlorhexidine disinfection protocol was compared in root canal treatment of apical periodontitis. A total of 60 permanent teeth were randomly allocated. Ozone gas (32 g m^{-3}) or NaOCl (3%) was applied, followed by dressing at an interval of 1 week ($Ca(OH)_2$). There were no significant differences between success rates between the ozone and NaOCl groups after 6/12 months. The most commonly found bacterial genera were *Streptococcus spp., Parvimonas spp.,* and *Prevotella spp*. Therefore, the ozone gas and NaOCl/chlorhexidine gluconate protocols used here showed no difference in bacterial reduction in the sampled areas of root canals [16].

Besides, a study evaluated the post-disinfection of the space using different irrigants. A total of 40 single-rooted mandibular premolars were collected and disinfected. The samples from Group 1 were submitted to photoactivated disinfection, group 2 was irrigated with ethanol-based propolis, group 3 was disinfected with ozone, and group 4 was irrigated with 2.25% NaOCl and 17% EDTA. Each sample was cut in 1 mm of coronal, middle, and apical and subjected to pushout bond strength (PBS) using a universal testing machine. The highest PBS at all three levels was found in group 4, channel disinfected with 2.5% NaOCl with 17% EDTA. Whereas, the lowest PBS was observed in group 1. In the intergroup comparison, the prosthetic space disinfected with propolis extract showed no significant difference compared to group 4 disinfected with 2.5% NaOCl with 17% EDTA and in all three levels. The intra-group comparison in all experimental groups showed no significant difference observed in the coronal and middle thirds of group 1, group 2, and group 3, respectively ($p>0.05$) [28].

Finally, a study evaluated the effectiveness of irrigation of periodontal pockets with ozonized water and 0.2% chlorhexidine gluconate as an adjunct to scaling and root planning in the management of chronic periodontitis. A total of 20 patients aged 30-60 years with chronic periodontitis were included. Irrigation was performed after 2 weeks of scaling and root planing on the same day with ozonized water and 0.2% chlorhexidine gluconate for two and a half minutes. Both groups showed improvement in clinical parameters. When the comparison was made between the two groups, the ozonized water showed a slightly better improvement than the chlorhexidine group. Therefore, subgingival irrigation with ozonized water is beneficial over conventional therapeutic modalities. Ozonized water restricts the formation of dental plaque and reduces the number of subgingival pathogens, thus treating periodontal diseases [29].

Bias and Limitations

No significant risks of bias were found with the use of ozone therapy in the processes of disinfection, accelerated healing, and cellular activations to prevent the progression of osteonecrosis of the jaw. The limitations found are related to the limited number of patients in each study, as well as the advances in phase III and IV clinical studies.

Conclusion

It was concluded that ozone therapy is an alternative to accelerate healing and reduce pain in traumatic and autoimmune ulcers. Ozone offers a painless alternative to conventional treatment for dental caries and has been shown to arrest primary root caries, primary pit, and fissure caries, and clinically reverse the lesion. Its antimicrobial action on endodontic microbiota is quite promising. Ozone gel is suggested as an adjunctive therapy in diabetic patients to improve periodontal health. Oxygen-ozone therapy has been

shown to enhance post-extraction healing in patients at risk for MRONJ. As for reducing the microbial load for patients undergoing root canal treatment, ozone therapy has inferior results when compared to conventional chemomechanical techniques using NaOCl.

CRediT

Author contributions: **Conceptualization-** Cristina Ponsoni, Maria Júlia Gonçalves, Andreia Borges Scriboni; **Formal Analysis-** Cristina Ponsoni, Maria Júlia Gonçalves; **Investigation-** Cristina Ponsoni, Maria Júlia Gonçalves; **Methodology-** Cristina Ponsoni, Maria Júlia Gonçalves, Andreia Borges Scriboni; **Project administration-** Cristina Ponsoni, Maria Júlia Gonçalves; **Supervision-**Andreia Borges Scriboni; **Writing - original draft-** Cristina Ponsoni, Maria Júlia Gonçalves, Andreia Borges Scriboni; **Writing-review & editing-** Cristina Ponsoni, Maria Júlia Gonçalves, Andreia Borges Scribonii.

Acknowledgment

Not applicable.

Ethical Approval

Not applicable.

Informed Consent

Not applicable.

Funding

Not applicable.

Data Sharing Statement

No additional data are available.

Conflict of Interest

The authors declare no conflict of interest.

Similarity Check

It was applied by Ithenticate®.

Peer Review Process

It was performed.

References

1. Veneri F, Filippini T, Consolo U, Vinceti M, Generali L. Ozone therapy in dentistry: An overview of the biological mechanisms involved (Review). Biomed Rep. 2024 Jun 12;21(2):115. doi:10.3892/br.2024.1803.
2. International Scientific Committee of Ozone Therapy-ISCO3: Learning methodology instructions and perfection in ozone therapy for medical doctors, 2015. Available online at: https://isco3.org/wp-content/uploads/2015/09/ISCO3-HUM-00-01.pdf.
3. World Federation of Ozone Therapy: WFOT'S review on evidence based ozone therapy, 2015. Available online at: https://spozonoterapia.pt/wpcontent/uploads/2016/02/WFOT-OZONE-2015-ENG.compressed.pdf.
4. Makeeva MK, Daurova FY, Byakova SF, Turkina AY. Treatment of an EndoPerio Lesion with Ozone Gas in a Patient with Aggressive Periodontitis: A Clinical Case Report and Literature Review. Clin Cosmet Investig Dent. 2020 Oct 28;12:447-464. doi: 10.2147/CCIDE.S267933.
5. Sen S, Sen S. Ozone therapy a new vista in dentistry: integrated review. Med Gas Res. 2020 Oct-Dec;10(4):189-192. doi: 10.4103/2045-9912.304226.
6. A SR, Reddy N, Dinapadu S, Reddy M, Pasari S. Role of ozone therapy in minimal intervention dentistry and endodontics - a review. J Int Oral Health. 2013 Jun;5(3):102-8. Epub 2013 Jun 23. PMID: 24155611; PMCID: PMC3769872.
7. Halbauer K, Prskalo K, Janković B, Tarle Z, Pandurić V, Kalenić S. Efficacy of ozone on microorganisms in the tooth root canal. Coll Antropol. 2013 Mar;37(1):101-7. PMID: 23697257.
8. Vincent JL, Lartigau G. A propos de l'ozone [Ozone]. Rev Fr Odontostomatol. 1971 Aug-Sep;18(7):839-58. French. PMID: 4945222.
9. Brignardello-Petersen R. Insufficient evidence regarding the effects of ozone when used as a disinfectant in root canal therapy. J Am Dent Assoc. 2020 May;151(5):e43. doi: 10.1016/j.adaj.2019.12.007. Epub 2020 Mar 24. PMID: 32220347.
10. Boch T, Tennert C, Vach K, Al-Ahmad A, Hellwig E, Polydorou O (2016) Effect of gaseous ozone on Enterococcus faecalis biofilm-an in vitro study. Clinical Oral Investigations, 20, 1733–9.

11. Case PD, Bird PS, Kahler WA, George R, Walsh LJ (2012) Treatment of root canal biofilms of Enterococcus faecalis with ozone gas and passive ultrasound activation. Journal of Endodontics 38, 523–6.
12. Rojas-Valencia MN (2011) Research on ozone application as disinfectant and action mechanisms on wastewater microorganisms. In: Mendez-Vilas A, ed. Science Against Microbial Pathogens: Communicating Current Research and Technological Advances. Badajoz: Formatex, pp. 263–71.
13. Junior OO, Lages GC (2012) Ozone therapy for lumbosciatic pain. Revista Dor 13, 261–70.
14. Hubbezoglu I, Zan R, Tunc T, Sumer Z (2014) Antibacterial efficacy of aqueous ozone in root canals infected by Enterococcus faecalis. Jundishapur Journal of Microbiology 7, e11411.
15. Huth KC, Quirling M, Maier S, et al. (2009) Effectiveness of ozone against endodontopathogenic microorganisms in a root canal biofilm model. International Endodontic Journal, 42, 3–13.
16. Kist S, Kollmuss M, Jung J, Schubert S, Hickel R, Huth KC (2017) Comparison of ozone gas and sodium hypochlorite/chlorhexidine two-visit disinfection protocols in treating apical periodontitis: a randomized controlled clinical trial. Clinical Oral Investigations 21, 995–1005.
17. Kaya BU, Kececi AD, Guldas HE, et al. Efficacy of endodontic applications of ozone and low-temperature atmospheric pressure plasma on root canals infected with Enterococcus faecalis. Letters in Applied Microbiology. 2014, 58,8–15.
18. Maglia DR, Souza BDAF, Visioli F. Efficacy of ozone therapy for oral mucosa wound healing: a systematic review and meta-analysis. Clin Oral Investig. 2024 Aug 17;28(9):490. doi: 10.1007/s00784-024-05873-2.
19. Hashim NT, Babiker R, Dasnadi SP, Islam MS, Chaitanya NC, Mohammed R, Farghal NS, Gobara B, Rahman MM. The Impact of Ozone on Periodontal Cell Line Viability and Function. Curr Issues Mol Biol. 2025 Jan 23;47(2):72. doi: 10.3390/cimb47020072.
20. Barahim AA, Shemais N, Mousa A, Darhous M. Clinical and radiographic evaluation of non-surgical therapy with and without ozone gel application in controlled type 2 diabetic patients with periodontitis: a randomized controlled clinical trial. BMC Oral Health. 2024 Nov 25;24(1):1435. doi: 10.1186/s12903-024-05212-7.
21. Scribante A, Gallo S, Pascadopoli M, Frani M, Butera A. Ozonized gels vs chlorhexidine in non-surgical periodontal treatment: A randomized clinical trial. Oral Dis. 2024 Sep;30(6):3993-4000. doi: 10.1111/odi.14829.
22. Di Fede O, La Mantia G, Del Gaizo C, Mauceri R, Matranga D, Campisi G. Reduction of MRONJ risk after exodontia by virtue of ozone infiltration: A randomized clinical trial. Oral Dis. 2024 Nov;30(8):5183-5194. doi: 10.1111/odi.15006.
23. Nagayoshi M, Kitamura C, Fukuizumi T, Nishihara T, Terashita M. Antimicrobial effect of ozonated water on bacteria invading dentinal tubules. Journal of Endodontics. 2004, 30, 778–81.
24. Hyslop PA, Hinshaw DB, Halsey WA Jr, et al. (1988) Mechanisms of oxidant-mediated cell injury. The glycolytic and mitochondrial pathways of ADP phosphorylation are major intracellular targets inactivated by hydrogen peroxide. The Journal of Biological Chemistry 263, 1665–75.
25. Filippi A (2001) The effects of ozonized water on epithelial wound healing (in German). Deutsche Zahna¨rztliche Zeitschrift 56, 104–8.
26. Noites R, Pina-Vaz C, Rocha R, Carvalho MF, Goncalves A, Pina-Vaz I (2014) Synergistic antimicrobial action of chlorhexidine and ozone in endodontic treatment. BioMed Research International 2014, 592423.
27. Silva EJNL, Prado MC, Soares DN, Hecksher F, Martins JNR, Fidalgo TKS. The effect of ozone therapy in root canal disinfection: a systematic review. Int Endod J. 2020 Mar;53(3):317-332. doi: 10.1111/iej.13229. Epub 2019 Nov 3. PMID: 31587303.
28. Almadi K, Alkahtany M, Alamam Y, Alaql F, Alaqil A, Almutairi M, Thafrah SMB. Influence of Propolis, Ozone and Photodynamic therapy in root canal disinfection on resin bond strength to radicular dentin. Photodiagnosis Photodyn Ther. 2021 Mar;33:102131. doi: 10.1016/j.pdpdt.2020.102131.
29. Kaur A, Bhavikatti SK, Das SS, Khanna S, Jain M, Kaur A. Efficacy of Ozonised Water and 0.2% Chlorhexidine Gluconate in the Management of Chronic Periodontitis when Used as an Irrigant in Conjugation with Phase I Therapy. J Contemp Dent Pract. 2019 Mar 1;20(3):318-323. PMID: 31204324.

MetaScience Press
The Best Science
https://zotarellifilhoscientificworks.com/