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# Effect of ozone application on carious lesions

## An integrative systematic review

Maud Isanine Fuchs

Dissertação conducente ao Grau de Mestre em Medicina Dentária (Ciclo Integrado)

Gandra, 17 de maio de 2022

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Trabalho realizado sob a Orientação de Prof. Doutor Arnaldo Sousa

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RESUMO

**Introdução:** A cárie dentária é uma doença multifatorial preocupante caracterizada pela desmineralização do dente. O tratamento tradicional muitas vezes traz medo e dor, por isso abordagens minimamente invasivas estão a crescer em popularidade para preservar a estrutura dentária. O ozono, um poderoso agente oxidante e antibacteriano, pode eliminar microrganismos envolvidos na lesão cariosa, impedindo o seu progresso e levando à paragem (controle) da infeção cariosa.

**Objetivo:** Avaliar o efeito clínico e antibacteriano do ozono nas lesões cariosas em comparação com o tratamento atual.

**Materiais e métodos:** A pesquisa foi realizada no Pubmed entre 2012 e Fevereiro de 2022, incluindo ensaios clínicos e ensaios controlados randomizados. Dos 33 artigos iniciais, 10 foram incluídos na revisão sistemática.

**Resultados:** Os estudos demonstraram que a aplicação de gás de ozono em lesões cariosas aumentou a remineralização, diminuindo a progressão da cárie, e teve um efeito antibacteriano importante para melhorar o sucesso do tratamento minimamente invasivo da cárie. No entanto, o efeito do ozono só atinge a parte externa da lesão.

**Conclusão:** O uso de ozono, um tratamento minimamente invasivo e indolor, como suporte ao tratamento clínico da cárie é promissor, mas mais estudos devem ser realizados para confirmar o potencial do ozono e determinar com mais precisão o tempo certo de aplicação e a concentração.

**Palavras-chaves:** *"Dentisteria, ozono, cáries dentárias, remineralização dentária"*

## ABSTRACT

**Introduction:** Dental carie is a preoccupying multifactorial disease characterized by the demineralization of the tooth. The traditional treatment often brings fear and pain so that minimally invasive approaches to preserve tooth structure are growing in popularity. Ozone, a powerful oxidant, and antibacterial agent, can eliminate microorganisms involved in the carious lesion, preventing its progress, and leading to carious arrest.

**Objective:** To assess the clinical and antibacterial effect of ozone on carious lesions compared to current carie treatment.

**Materials and methods:** The research was carried out on PubMed between 2012 and February 2022 including randomized controlled trials and clinical trials. From the initial 33 articles, 10 were included in the systematic review.

**Results:** The studies demonstrated that ozone gas application on carious lesions enhanced remineralization, decreasing the progression of the carie, and had an important antibacterial effect to improve the success of minimally invasive carie treatment. However, ozone's effect only reaches outer parts of the lesion.

**Conclusion:** As a support to carie clinical care, the use of ozone, a minimally invasive, painless, and rapid treatment, is promising. Nevertheless, more studies with similar protocols must be performed to confirm ozone's potential and determine more precisely the optimal duration of application and concentration.

**Keywords:** *"Dentistry, ozone, dental caries, tooth remineralization"*

## General Index

## Figure and table index

## List of abbreviations

pH- potential of hydrogen

PRISMA- Preferred Reporting Items for Systematic Reviews and Meta-Analyses

RCT- Randomized Controlled Trial

CT- Clinical Trial

CHX- Chlorhexidine

$Ca(OH)^2$- calcium hydroxide

*Spp*- species pluralis

ACP- Amorphous Calcium Phosphate

*S. mutans- Streptococcus mutans*

*L. casei- Lactobacillus casei*

Ppm- parts per million

## 1- INTRODUCTION

Dental carie is a very old and well-known multifactorial disease that is characterized by destruction of the tooth. It mainly involves two groups of bacteria, the *Streptococcus mutans* and the *Lactobacillus* species, which can produce acids through the metabolization of carbohydrates presented in the host's mouth. These acids lead to a decrease of the pH below its critical value (5.5) and result in hydroxyapatite dissolution of the enamel. It starts with a small surface demineralization and can progress to cavitation and even pulp involvement if not treated (1-3).

The conventional carie treatment is based on drilling the affected tissue with a rotatory instrument and then filling the cavity with restorative materials such as resin composite or amalgam. This method known as complete carie removal is not only often associated with pain, discomfort, and fear but it also increases the chances of pulp exposure, postoperative pain, and undermining of the tooth structure (4-6).

In order to overcome those disadvantages, the use of minimally invasive approach is growing in popularity, with the objective to maximally preserve the tooth structure. For non-cavitated carie lesions, it is essential to revert the process by remineralizing the tooth surface via fluoride or with a sealant (7). Indeed, retained sealants block the nutrient absorption from the oral cavity, thus preventing the bacteria's cariogenic potential and arresting the lesion (8).

Among these interventions to treat deep cavitated lesions, the two most common are the partial carie removal and the stepwise excavation. The former can eliminate the superficial carie in a single visit while the latter takes two a few months apart (6, 7). The two-stage excavation procedure permits inactivation of the carie (harder and drier) and the promotion of tertiary dentin after the first excavation which considerably decrease the risk for pulp exposure during the final excavation (9). Partial carie removal provides the same advantages except that it is done in a single session so that the risk of pulp exposure with the re-entry is non-existent (7).

Pharmaceutical approaches are nowadays growing its utilization as an additional method to stop carie progression. After the carie excavation, the remaining bacteria on cavity walls must be eliminated by antibacterial agents to ensure proper disinfection before the restoration, and success of the treatment (10). Chlorhexidine is the most common and known antibacterial agent but in recent years, ozone was found to be promising as well (11).

Ozone is a gas molecule composed of three oxygen atoms which is found in abundance in our atmosphere. Its importance comes from its ability to absorb ultraviolet rays and therefore protect us from them (12, 13). Nowadays, ozone therapy is used in dentistry for various treatments in periodontology, endodontics, dental bleaching, oral medicine, and carie prevention and management (14, 15).

The application of ozone on oral tissue is achieved in the form of ozonated water, ozonated oil, or ozone gas (16). In addition to being non-invasive and painless, which increases the patient's acceptability, it is also a powerful oxidant and antibacterial agent. Indeed, ozone has the ability to destroy the cell walls and the cytoplasmic membranes of microorganisms by oxidation. The permeability is damaged, and ozone can easily degrade glycoproteins, glycolipids, and other amino acids (17).

Thus, theoretically, ozone application could eliminate microorganisms involved in the carious lesion, preventing its progress, and leading to carious arrest. However, this requires to be confirmed by more research.

## 2- OBJECTIVE

The aim of this systematic review is to assess the clinical and antibacterial effect of ozone application on carious lesions compared to conventional carie treatment.

## 3- MATERIALS AND METHODS

In this systematic review, the methodological protocol applied was the one recommended in the PRISMA Statement, following the PRISMA checklist and using the PRISMA flow diagram.

### 3.1 Eligibility criteria

The PICO strategy ("Patient, Intervention, Comparison, Outcome") was used to define the guiding question for this integrative systematic review:

-Patient, population, or problem

In vitro or in vivo studies concerning any type of carious lesions that don't involve the pulp

-Intervention or exposure

Ozone application on carious lesion

-Comparison

Conventional carie treatment

-Outcome

Clinical and microbiological evolution, benefits, and viability

The question thus became: does ozone application provides benefits in carie treatment compared to a conventional treatment?

The inclusion criteria considered were the following:

- Articles published from 2012 to February 2022 (10 years)
- English or Portuguese languages
- Randomized controlled trials or clinical trials

The exclusion criteria were the following:

- Articles published before 2012
- Systematic reviews, meta-analyses, reviews
- Articles without abstract

- Articles without full text available
- Articles in another language than English or Portuguese
- Articles referring to periodontology, surgery, endodontics, tooth bleaching instead of carious lesion

3.2 Search strategy

The research was carried out on PubMed (via the National Library of Medicine) using the following advanced search builder and Mesh Terms: ("Ozone"[Mesh] AND "Dental Caries"[Mesh]) OR ("Dentistry"[Mesh] AND "Ozone/therapeutic use"[Mesh]) OR ("Ozone"[Mesh] AND "Tooth Remineralization"[Mesh]).

The title and abstract of each potentially appropriate article were first analyzed. The selected ones, which met the inclusion criteria, were then entirely read, and assessed for eligibility.

After completing the final article selection, all relevant data were extracted and organized in a table. The important information picked from the articles were author's names, publication dates, study designs, objectives, participants, comparisons, results, and conclusions.

# 4- RESULTS

## 4.1 Study selection

The bibliographic search on PubMed with the date and study design restrictions and with the Mesh Terms combination gave a first result of 33 articles. No duplicate was found. From those 33 articles, 22 were excluded after title and abstract analysis because they didn't meet this systematic review's objective. 11 articles met the inclusion criteria and were identified for full text assessment. After a meticulous reading of the 11 articles, a total of 10 articles were selected and included in the integrative systematic review. The search strategy is presented in the PRISMA flow diagram.

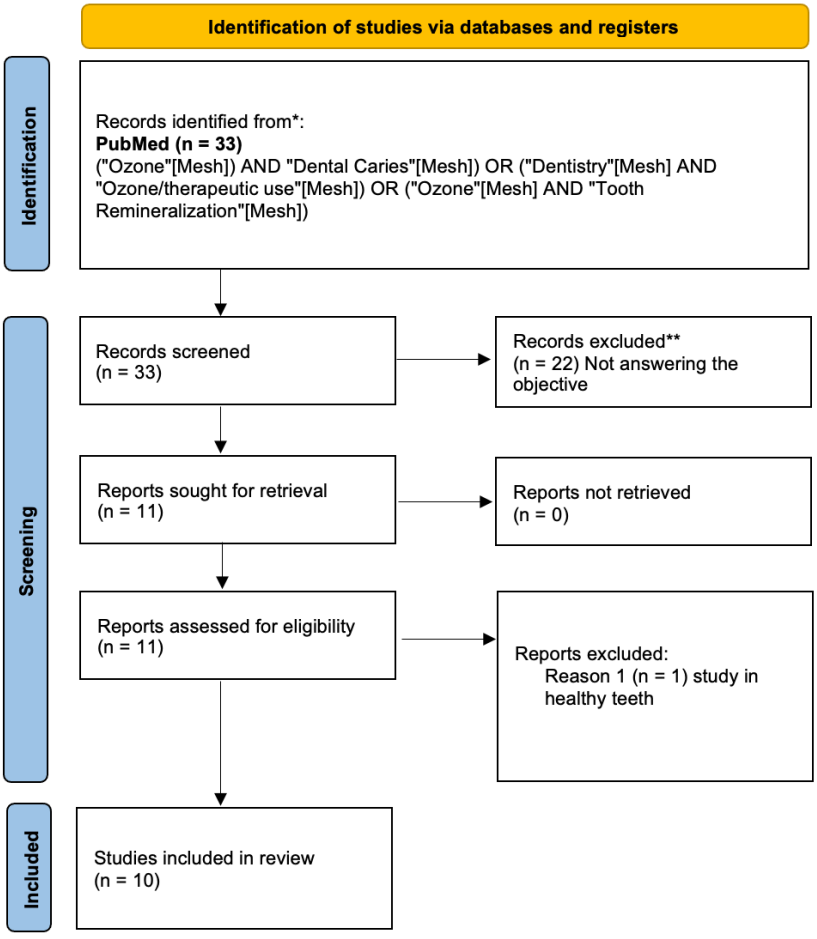


Figure 1: Flow diagram of the search strategy used in the review

## 4.2 Study characteristics

All data such as author's names, publication dates, study designs, objectives, participants, comparisons, results, and conclusions, from the 10 selected articles, were retrieved and presented in the following table.

| Authors, date, and study design | Objectives | Participants | Comparisons | Results | Conclusions |
|---|---|---|---|---|---|
| Durmus N, Tok Y, Kaya S et al.<br><br>2019<br><br>RCT | To evaluate the effect of ozone application in the two-visit indirect pulp therapy, clinically (color, consistency, humidity of the dentin) and microbiologically, and to compare the results with CHX and without any cavity disinfectant application. | 105 patients aged between 6 and 13 years old with almost exposed deep carious lesions in first permanent molars. | Group 1 (n=35): two-visit indirect pulp therapy without any disinfectant (control group).<br><br>Group 2 (n=35): two-visit indirect pulp therapy with 60s CHX application (positive control group).<br><br>Group 3 (n=35): two-visit indirect pulp therapy with 60s gaseous ozone application (experimental group).<br><br>Each group had $Ca(OH)^2$ liner and were temporary sealed with glass ionomer cement.<br><br>Dentin samples were collected from each group at each step. | The dentin became harder, drier, and darker in all groups. However, the CHX and ozone groups were statistically better in terms of hardness and dryness.<br><br>There were 79.11%, 98.39%, and 93.33% bacterial reduction in the control, positive control, and experimental groups, respectively, between first excavation and after 4 months. But after the second excavation there was no significant difference.<br><br>2% CHX showed significantly higher reduction (90,32%) compared to the gaseous ozone application (53,42%) just after its application. | The two-visit indirect therapy provided successful results in reducing the number of microorganisms and making decay arrested. Moreover, disinfectant application might support these effects and increase the treatment success. In addition, having both higher antimicrobial effects and easy to obtain, CHX would be more convenient in the two-visit indirect pulp therapy. |
| Krunić J, Stojanović N, Dukić L et al.<br><br>2019<br><br>RCT | To evaluate local effect of gaseous ozone on bacteria in deep carious lesions after incomplete carie removal. | 48 patients aged 20–48, with primary carious lesions on occlusal or proximal surface. | Group 1 (n=24): 40s ozone disinfection<br><br>Group 2 (n=24): 60s CHX disinfection<br><br>In both groups, pulpal wall was lined with $Ca(OH)^2$ base material and cavity was sealed with temporary filling. | Ozone and CHX application significantly decreased the number of total bacteria by 68% and 34.5%, respectively.<br><br>Ozone and CHX application significantly decreased counts of *Lactobacillus spp.* by 30% and 66%, respectively. | Local application of ozone in deep carious lesions after incomplete dentin carie removal provides significant antibacterial effect measured by total number of bacteria and *Lactobacillus spp.* |
| Oztas N, Unal M<br><br>2015<br><br>RCT | To evaluate the remineralizing capacity of fissure sealants used with gaseous ozone and their clinical success during a 12 months period. | 60 patients between 7 and 9 years old with non-cavitated first permanent molars but having initial pit and fissure caries on enamel. | Group 1 (n=20):<br>40s ozone application on one side of each tooth followed by Aegis ACP sealant application on both sides.<br><br>Group 2 (n=20):<br>40s ozone application on one side of each tooth followed by Fuji Triage sealant application on both sides.<br><br>Group 3 (n=20): | The results showed that, when initial and 12th month DIAGNOdent values were compared, a decrease was seen in all three groups (with ozone), but the difference was statistically significant only for group 1 and 2.<br><br>In the control group teeth, when the initial and 12th month DIAGNOdent values were statistically assessed, the differences were found to be | Gaseous ozone application before applying fissure sealant did not affect the clinical success of the Aegis, Fuji Triage and Helioseal fissure sealants.<br>Gaseous ozone may be helpful together with fissure sealants with ACP or fluoride content for initial |

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| | | | 40s ozone application on one side of each tooth followed by Helioseal sealant application on both sides. | statistically insignificant in all three groups. | caries remineralization. |
| Grocholewicz K, Matkowska-Cichocka G, Makowiecki P et al.<br><br>2020<br><br>RCT | To assess and compare the efficacy of nano-hydroxyapatite gel and gaseous ozone therapy on enamel remineralization of initial approximal caries lesions of premolar and molar teeth in adults. | 546 lesions were selected in patients aged from 20 to 30 years with initial approximal caries. | Group 1 (n= 178): remineralizing gel containing 10% of nanohydroxyapatite.<br><br>Group 2 (n= 205): ozone application at the first appointment, after 2, 4 and 6 months.<br><br>Group 3 (n= 163): combination of the remineralizing gel and ozone therapy. | After 1 year of observation, there were 36,5%, 60%, and 69,3% of carie regression in group 1, 2, and 3 respectively.<br><br>After 2 years findings were like those in the 1-year follow-up. The smallest rate of caries reversal was observed in group 1 (18.0%). In group 2 it was 38.0%, and in group 3 45.4%. | Nano-hydroxyapatite gel and ozone therapy show some properties to remineralize initial approximal enamel and dentine subsurface lesions. The combination of both methods produces the best effect compared to nano-hydroxyapatite or ozone therapy alone. |
| Yazıcıoğlu O, Ulukapı H<br><br>2014<br><br>RCT | To examine the effectiveness of ozone as a non-invasive treatment on the non-cavitated approximal caries lesions over an 18-month period *in vivo.* | 60 non-cavitated approximal caries lesions of the molar or premolar teeth. | Group 1 (n=33): nothing was applied in the control group<br><br>Group 2: not relevant<br><br>Group 3 (n= 27): gaseous ozone was applied once for 40s to the approximal surfaces.<br><br>Group 4: not relevant | In the control group, the visual, radiographic examination and DIAGNOdent readings showed progression of the dentine lesions after 18 months.<br><br>For the ozone group, scores of the inner half of the enamel lesions increased, but the scores of the outer half of the enamel lesions were stable. | Ozone gas application removed the microorganisms in the outer half of enamel lesions and stopped the demineralization activity. However, ozone gas application couldn't remove the microorganisms in deeper lesions. |
| Al-Omiri M, Alqahtani N, Alahmari N et al.<br><br>2021<br><br>RCT | Measure the need for root canal treatment following 20s of ozone application on partially removed deep caries in comparison with total caries removal and restoration. | 84 participants between 20 and 27 years old with an almost exposed extensive carious lesion in a molar. | Group 1 (n=42): partial carie removal (1 mm thickness left on the pulpal floor). Then, ozone gas application for 20s.<br><br>Group 2 (n=42): total carie removal<br><br>Both groups were then covered by a glass ionomer cement and a composite restoration. | After 2 months, 8 (19.0%) participants required root canal treatment from the traditional management group whilst 1 (2.4%) participant required root canal treatment in the ozone group.<br><br>After 2 years follow-up, the same numbers of participants in both groups required root canal treatment. | In deep carious lesions near the pulp, 20 s of ozone application on partially removed deep caries before a restoration would lead to less need for endodontic treatment in comparison to a conventional treatment protocol of total caries removal and a restoration. |
| Mese M, Tok Y, Kaya S et al.<br><br>2020<br><br>RCT | To assess the antibacterial and clinical effects (color, consistency, humidity of the dentin) of ozone application before temporary restoration in the stepwise excavation procedure. | 105 patients aged 6-10 years with deep carious lesions involving occlusal and/or occluso-proximal surfaces in primary molars. | Group 1 (n=35): stepwise excavation with no disinfectant.<br><br>Group 2 (n=35): stepwise excavation with 60s CHX disinfection.<br><br>Group 3 (n=35): stepwise excavation with 60s gaseous ozone disinfection.<br><br>Each group had $Ca(OH)^2$ liner and were temporary sealed with glass ionomer cement.<br><br>Dentin samples were collected from each group at each step. | Dentin became harder and dryer in all the groups, and darker only with CHX and ozone, without significant difference between them.<br><br>There were 74.18%, 93.25%, and 82.29% bacterial reduction in the control, positive control (CHX), and experimental (ozone) groups, respectively, between the first excavation and after 4 months. But after the second excavation, it showed no significant difference among group 1, 2, and 3 (98.43%, 99.78%, and 98.66%, respectively). | Cavity disinfectants in the stepwise excavation reduce bacterial levels in the cavity, which may allow the avoidance of the second step of the procedure for primary teeth.<br><br>Both CHX and ozone application were effective in bacterial reduction in the stepwise excavation procedure, with the CHX application found to be superior to ozone gas. |

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| Safwat O, Elkateb M, Dowidar K et al.<br><br>2018<br><br>CT | To assess the microbial effect of ozone gas on dentinal lesions in young permanent molars using the stepwise excavation. | A sample of 80 bilateral immature first permanent molars, with deep class I carious lesions in 40 healthy children aged 7-9 years. | Group 1 (n= 40): stepwise excavation followed by 40s ozone gas disinfection.<br><br>Group 2 (n= 40): stepwise excavation followed by $Ca(OH)^2$ base material.<br><br>Dentin samples were collected from each group at each step. | The reduction of S. mutans, Lactobacillus and Candida were respectively 65%, 51.61% and 74.21% after direct application of ozone, 97.16%, 91.13% and 100% after 6 months and 91.73%, 74.31% and 100% after 12 months.<br><br>No significant differences were found between both groups after 6 or 12 months in the S. mutans, Lactobacillus and Candida. | Ozone gas application for 40 seconds via the HealOzone device had a significant antimicrobial effect especially against S. mutans, in deep class I carious lesions.<br><br>The application of ozone, or $Ca(OH)^2$ have comparable effect on affected dentin in deep cavities. |
| Polydorou O, Halili A, Wittmer A et al.<br><br>2012<br><br>RCT | To evaluate the antimicrobial effect of ozone application on the two most important cariogenic bugs, S. mutans and L. casei, 4 and 8 weeks after the treatment, using a tooth cavity model. | 48 extracted human non-carious third molars in which 3 cavities were prepared (one used for the evaluation of dentine infection and the 2 others were used as experimental cavities). | Group A (n= 12): S. mutans incubation without treatment<br><br>Group B (n= 12): S. mutans incubation with 60s ozone treatment<br><br>Group C (n= 12): L. casei incubation without treatment<br><br>Group D (n= 12): L. casei incubation with 60s ozone treatment | For S. mutans, a significant difference was found between the control and treated groups after 4 and 8 weeks storage. No significant difference was found between 4 and 8 weeks.<br><br>For L. casei, the treatment by ozone did not show any significant effect. The storage time did not have any significant influence on the effect of ozone. | Ozone has some antibacterial effect on S. mutans, with stable effect through the period of 8 weeks. However, it is doubted that such an effect is enough for it to be used alone as an antibacterial method. In contrast, L. casei was not affected by ozone under the present condition. |
| Safwat O, Elkateb M, Dowidar K et al.<br><br>2017<br><br>CT | To evaluate the clinical changes in dentin of deep carious lesions in young permanent molars following ozone application with and without the use of a remineralizing solution using the stepwise excavation. | A sample of 162 bilateral immature first permanent molars, with deep class I carious lesions in 40 healthy children aged 7–9 years. | Group 1 (n=80):<br>Subgroup 1a (n=40): stepwise excavation followed by 40s ozone gas disinfection (experimental group).<br><br>Subgroup 1b (n=40): stepwise excavation followed by $Ca(OH)^2$ base material (control group)<br><br>Group 2 (n=82): Subgroup 2a (n=41): Carious lesions were exposed to ozone gas followed by the application of a remineralizing solution.<br><br>Subgroup 2b:<br>stepwise excavation followed by $Ca(OH)^2$ base material (control group)<br><br>Color, consistency, and DIAGNOdent readings were assessed at each step for each group. | Differences of dentin color and consistency between baseline and after 6 or 12 months was not statistically significant after ozone application.<br><br>In subgroup 1a and 2a, there was no significant difference between base line DIAGNOdent reading and directly after application of ozone, whereas the difference was statistically significant between the reading directly following ozone treatment and after 6 months. No significant differences were found between DIAGNOdent readings at different evaluation time for 12-months follow-up cases.<br><br>In control subgroups, there was no significant differences between all evaluation time. | Ozone application through the stepwise excavation had no significant effect on dentin color, and consistency in young permanent molars. DIAGNOdent was unreliable as a diagnostic tool in monitoring caries activity, following treatment of deep dentinal lesions because when values are higher than 50 the rates of bacterial detection are 100%. |

Table 1: Characteristics of the articles included in the systematic review

# 5. DISCUSSION

## 5.1 Delivery device, ozone safety

In the present systematic review, 2 studies used in their experimental protocols the OzonyTron open device, and 8 the HealOzone closed device. These are the 2 most well-known and commercialized ozone delivery systems in dentistry.

The HealOzone device releases ozone gas at a high concentration of 2100 ppm ± 200 ppm in the designated tooth. To treat dental carie with such a high concentration without damaging the whole mouth, ozone is applied via a closed device. Silicone cups with different sizes permit a good sealing of the target tooth, avoiding ozone leakage. At the end of the application, the remaining ozone is sent back to the device to be decomposed. The inconvenience is that the size of the equipment needed to ensure safety doesn't allow access to all surfaces (18,19).

The OzonyTron, on the contrary, is an open device using plasma-electrodes to apply ozone. The concentration used is lower, around 525 ppm, which is much safer in case of leakage (19).

Among the studies included in our review, only one specifically reported no side effect (19). The others didn't relay anything about adverse effects of ozone in dental carie treatment.

## 5.2 Remineralizing capacity in initial carious lesions

Pit and fissure caries are very common non-cavitated enamel lesions affecting mainly molars. It is important to try to preserve the maximum tooth integrity. While there are non-cavitated, the objective is to reverse the balance of demineralization and enhance remineralization of the affected tissue of all initial carious lesions. To treat them, Oztas N et al. (20) proposed to expose the pit and fissure lesions to ozone during 40s prior to 3 different fissure sealants application. This minimally invasive treatment demonstrated that, ozone not only doesn't interfere with the success of the sealant, but it also triggers the

remineralization of the initial carie. This factor wasn't significant when all sealants were applied alone. The best results in initial carie remineralization were achieved with the association of ozone and sealants containing amorphous calcium phosphate or fluoride. Gaseous ozone might play an important role for non-cavitated early carie treatment.

Approximal lesions, by their location, make them difficult to treat without damaging unnecessary surrounding tissue. The study of Grocholewicz K et al. (21) showed that ozone alone had the potential to act on the remineralization of initial approximal lesions but its combination with 10% nano-hydroxyapatite gel improved the carie regression even more. Ozone was applied at the first appointment, after 2, 4 and 6 months in the study. With a sufficient time of treatment, combining ozone and the nano-hydroxyapatite gel could reverse the process of demineralization, cure the carious initial lesion, and avoid the restorative and traumatic conventional treatment

While supporting the ability of ozone gas to arrest demineralization and promote remineralization, the Yazıcıoğlu O et al. controlled trial (22) also demonstrated that its efficacity on approximal initial lesions was limited. Indeed, ozone application removed the cariogenic bacteria and stopped the demineralization in the external part of the lesion, but regarding the deeper part, no evolution was seen after 18 months.
It shows that the application of ozone after cavity preparation, when only one thick layer of carious dentine was left, could destroy the residual microorganisms in the cavity and increase the success of the treatment.

## 5.3 Cavity disinfectant in deep carious lesions

### 5.3.a After incomplete carie removal

The traditional way to treat deep carious lesions is to remove all carious tissue with rotatory and other invasive instruments. This method is traumatic and can easily remove healthy tissue, increasing at the same time the risk to reach the dental pulp.
Knowing the antibacterial potential of ozone, Al-Omiri M et al. (23) compared the need for endodontic treatment after total carie removal with partial carie removal followed by ozone application for 20s. A thin layer of carious tissue was left on the pulpar floor, and then ozone was used as cavity disinfectant to eliminate residual bacteria. After 2 years, the participants who had total carie removal needed much more endodontic treatment than the ones treated with partial carie removal and ozone. Ozone has proven to be effective as a conservative carie treatment thanks to its ability to destroy residual microorganisms.

Krunić J et al. (19) further evaluated this effect specifically on *Lactobacillus ssp*, one of carie's most common bacteria. The results are in line with the Al-Omiri M study (23): local application of ozone for 40s significantly decreased total bacteria and *Lactobacillus ssp* counts after incomplete carie removal similarly to a 60s chlorhexidine application.
However, if, on the one hand, it shows that ozone is more effective in reducing the total number of bacteria (68% vs 34,5%), on the other, chlorhexidine performs better on the reduction of the number of *Lactobacillus* spp (66% vs 30%).

Ozone gas showed a promising antibacterial effect against cariogenic bacteria when applied after an incomplete carie removal. Its application permits a good disinfection of the superficial layer in a single visit, leading to better chances of success and less risk of pulpal involvement.

5.3.b After stepwise excavation

Another alternative to conventional carie treatment is the stepwise excavation, also known as two-step indirect pulp therapy, used whenever there is a deep carious lesion associated with pulp perforation risk. During the first excavation, the upper part of the infected tissue is removed with a dentine excavator and then the cavity is sealed with a temporary restoration for a period of 3 to 6 months. After that time, the cavity is reopened, and the remaining demineralized dentin is eliminated. The objective of this two-step procedure is to stop carie progression by isolating it from the oral environment, and to induce the formation of tertiary dentin, decreasing the risks of pulp exposure during the second excavation. In order to ensure a proper cavity disinfection, the killing of the remaining bacteria and the promotion of the remineralization after the first excavation, different authors are proposing the use of ozone gas as an adjuvant to the procedure.

In a recent study, Durmus N et al (18) compared the antibacterial effect of ozone, chlorhexidine, and no disinfection in the stepwise excavation. Chlorhexidine at 2% is known to be powerful against Gram-positive and Gram-negative microorganisms, yeast, and fungi. It is the antibacterial most used in cavity disinfection. Ozone also has the ability to act on microorganisms and fungi by destroying the cell walls and oxidizing the cytoplasmic membranes (18). After 4 months, all groups had decreased levels of bacteria. 60s of chlorhexidine disinfection had the best results (98% of bacterial reduction), followed by 60s of ozone disinfection (93% of bacterial reduction). However, after the second excavation, there were no significant difference between them. It means that the use of a cavity disinfectant is very successful and could remove the need of the second excavation. Ozone gas is effective as antibacterial agent even if chlorhexidine seems superior. A year later, in 2020, Mese M et al. (24) did their study with the exact same conditions. Their results were practically the same: 93% of bacterial reduction for chlorhexidine application versus 82% for ozone after 4 months.
Both recent studies confirm the potential of ozone, almost as high as chlorhexidine, used as a support in carie treatment in order to improve the success of the procedure (18,24).

After the first excavation, the gold standard material to isolate residual bacteria is a $Ca(OH)^2$ liner. It is called indirect pulp capping and has proven to be effective in tertiary dentin formation and bacterial reduction in the remaining carie. Safwat O et al. (25) compared its already known antibacterial effect to 40s of ozone disinfection in young permanent molars treated with the stepwise excavation. 12 months after ozone application, the bacterial reduction was significant. Indeed, ozone gas permitted 91,73% reduction of *S. mutans*, and 74,31% reduction of *Lactobacillus*. Ozone antibacterial effect was especially demonstrated against *S. mutans* but with a noteworthy action on *Lactobacillus* species nonetheless. No significant difference between the $Ca(OH)^2$ liner and ozone disinfection were found in this study. The similar results of both procedures are leading one more time to the fact that the application of ozone inside a prepared cavity is an efficient alternative to the common treatment.

Its action against cariogenic bacteria, more precisely directed against *S. mutans*, was also recently highlighted by Polydorou O et al. (26). This *in vitro* study was realized with extracted molars in which cavity model were prepared. *S. mutans* or *L. casei* were inoculated in the cavities. Half of each group received 60s of ozone treatment and the other half had no treatment in comparison. After a period of 4 and 8 weeks, results showed a significant bacterial reduction of *S. mutans* in the ozone treatment group that seemed stable in time. However, the population of *L. casei* wasn't influenced by the presence of ozone neither after 4 weeks storage, nor after 8 weeks. These findings seem to coincide with the results of the Krunić J *in vivo* research cited above (20) in which ozone proved to be much more efficient against the total number of bacteria than against the *Lactobacillus* species specifically. The explanation may come from the fact that sucrose had been administered during the incubation, which resulted in a low pH that modified *L. casei* behavior in the study of Polydorou O (26).

The outcomes all agree to ozone's strong antibacterial effect against *S. mutans*, and more lightly against *Lactobacillus* species (19,26). However, further research, both *in vivo* and *in vitro*, are necessary to demonstrate more precisely the role of ozone against specific bacteria involved in the carie process.

## 5.4 Clinical changes with ozone application

The other objective of this systematic review is to assess dentine clinical changes after application of ozone in the cavity. There were only 3 research included in the review studying the possible dentine alterations in terms of color, consistency, and humidity. The first one, by Safwat O et al. in 2017 (27) evaluated ozone effect with and without a remineralizing solution during the stepwise excavation procedure. Even though the correlation between bacterial number and Diagnodent readings in this study wasn't reliable because of readings above 50, dentine color and consistency could be assessed after a period of 6 and 12 months. A small improvement in dentine hardness was reported even if there were no significant changes in the consistency and color between baseline and after 6 and 12 months. It is probably due to the benefits of the excavation and of the sealing of the cavity decreasing carie progression and initiating tertiary dentin formation. On the contrary, the recent studies of Durmus N et al. (18) and Mese M et al. (24) showed a notable increase of dentine hardness, and dryness in all the groups using either no disinfectant, chlorhexidine, or ozone in the stepwise excavation. The only difference between those 2 studies was regarding the color changes. Dentine became darker in all groups in Durmus N (18) results but only in chlorhexidine and ozone groups for Mese M (24). Once again, the first excavation and the sealed restoration played an important role in those results as explained above. However, a significant higher increase of hardness and decrease of dentine humidity was noticed after 4 months when the cavity had been disinfected with chlorhexidine or gaseous ozone (18). Therefore, the interest in ozone to assist the reverse of carie activity seems justified.

## 6- CONCLUSION

Throughout this systematic review, we've seen that, over the last 10 years, ozone has proven to be effective on carious lesions at all stages. Indeed, the strong remineralizing capacity of ozone gas applied to non-cavitated initial lesions was demonstrated. Moreover, its effectiveness is increased coupled with a remineralizing solution or a fissure sealant.

In addition, ozone's antibacterial action proved to be particularly successful in improving cavity disinfection and hardening of the dentine in advanced carious lesion. In fact, the effectiveness was similar to a $Ca(OH)^2$ liner or chlorhexidine disinfection and significantly higher compared to no disinfection of the prepared cavity.

Ozone must be used as a complement to a minimally invasive treatment mainly because its action is restricted to the outer part of the lesion. When well executed, this association achieves a less traumatic treatment than the traditional drilling and filling technique.

As support to carie clinical care, the use of ozone, a minimally invasive, painless, and rapid treatment, is promising. Nevertheless, more studies with similar protocols must be performed to confirm ozone's potential and determine more precisely the optimal duration of application and concentration.

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