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REVIEW ARTICLE

Ozone therapy in the management and prevention of caries

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The purpose of this article was to assess the effectiveness of ozone therapy in the management and prevention of caries, reviewing clinical and *in vitro* studies. Ozone has proven to be effective against gram-negative and gram-positive bacteria, viruses, and fungi. In dentistry, most of the published articles are based on ozone's antimicrobial effects and the treatment of caries. Most of the clinical studies reported ozone to be a promising alternative to conventional methods for caries management. However, a few studies have shown ozone to be insufficient for preventing caries and reducing microorganisms in open occlusal carious lesions. Ozone might be a useful tool to reduce and control oral infectious microorganisms in dental plaque and dental cavity. However, the results of *in vitro* studies are controversial; while some researchers reported that ozone therapy had a minimal or no effect on the viability of microorganisms, others suggested ozone to be highly effective in killing both gram-positive and gram-negative oral microorganisms. Therefore, more evidence is required before ozone can be accepted as an alternative to present methods for the management and prevention of caries.

Introduction

Dental caries is a bacterially mediated disease characterized by the demineralization of the tooth surface, which may lead to cavitation, discomfort, pain, and even tooth loss,[1] and it is a major oral health problem that affects 60–90% of schoolchildren and a vast majority of adults.[2,3] Bacteria play a very important role in the initiation and progression of carious lesions.[4,5] Reducing the levels of caries associated bacterial species in dental plaque is one of the preventive strategies to prevent the initiation of caries and to treat the disease.[6,7] Furthermore, to prevent secondary caries that may be related to the presence of residual bacteria under restorations,[8,9] the use of an antibacterial treatment after caries removal seems to be meaningful.[5] To arrest caries progression, several

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antibacterial treatments have been proposed in order to mechanically and/or chemically reduce biofilm formation[3,10–12] and reduce the amount of residual bacteria after caries removal.[5,13–15] Nowadays, to treat dental caries, pharmaceutical approaches have gained popularity. Such approaches give the opportunity of caries treatment without drilling. A novel concept ozone therapy has been introduced for the treatment of caries,[16,17] disinfection of the cavity,[18] and reduction in the levels of caries-associated microorganisms.[19,20]

Ozone is an energy-rich and highly unstable form of oxygen.[7,21] It is one of the most important gases in the stratosphere because of its ability to filter ultraviolet (UV) rays, which are crucial for the biological balance in the biosphere.[22] This allotropic molecule has been widely used as a treatment agent of more than 50 pathological processes[23–30] and has been used in dentistry.[31–33] Although there are relatively simple application forms and active mechanisms (Fig. 1), the use of ozone in dentistry developed very modestly.[34] Dr. Edward Fisch was the first dentist to use ozone in his practice and introduced it to the German surgeon Dr. Erwin Payr who used ozone from that time forward in surgery.[35] Because of the infectious diseases associated with the oral cavity, there has been interest in ozone use in dentistry due to its antimicrobial properties.[22,34] Ozone has been used in various fields in dentistry such as periodontology,[22] endodontics,[35] and maxillofacial surgery.[36] It has proved to be effective against gram-negative and gram-positive bacteria, viruses, and fungi.[37] Ozone's action is twofold but simultaneous; it generates direct molecular-level reactions in the medium in which it is released and indirectly destroys bacteria by the production of free radicals.[33] It is generally accepted that oxidation due to ozone starts the destruction of cell walls and cytoplasmic membranes of microorganisms; after the membrane is damaged, permeability increases and ozone molecules can easily enter into the cells.[12,38] In dentistry, most of the published articles are based on ozone's antimicrobial effects[18,19,39,40] and the treatment of caries.[20,41,42] The purpose of this article is to assess the effectiveness of ozone therapy in the management and prevention of caries, reviewing clinical and *in vitro* studies.

Clinical studies

Holmes[16] assessed the effect of an ozone delivery system, combined with the daily use of a remineralizing patient kit, on the clinical severity of noncavitated leathery primary root carious lesions (PRCLs), in an older population group. He concluded that dentistry has the ability to reverse lesions with just 40 seconds of ozone treatment. At 18 months, 100% reversal and remineralization had been achieved. Leathery noncavitated primary root caries can be arrested nonoperatively using ozone and remineralizing products. It is suggested that this treatment regime is an effective alternative to conventional "drilling and filling."

Baysan and Lynch[43] have evaluated the effect of ozone on the microbial flora and clinical severity of primary root caries *in vivo*. A biopsy was taken from half of each lesion prior to and after ozone application, and the microbiological counts are determined and compared. The authors reported that ozone application for either 10 seconds or 20 seconds dramatically reduced most of the microorganisms in primary root caries lesions without any side effects recorded at recall intervals between 3 months and 5.5 months.

The effect of ozone on noncavitated initial occlusal fissure caries lesions considering the patient's current caries risk has been investigated with a randomized controlled clinical study, and lesion progression or reversal was monitored by the laser fluorescence system DIAGNOdent for up to 3 months. The results of the study suggested that the application of ozone gas on noncavitated occlusal fissure caries lesions has an improving effect in patients at high caries risk. The authors suggested ozone as a novel therapeutic approach to the management of noncavitated initial fissure caries lesions.[44]

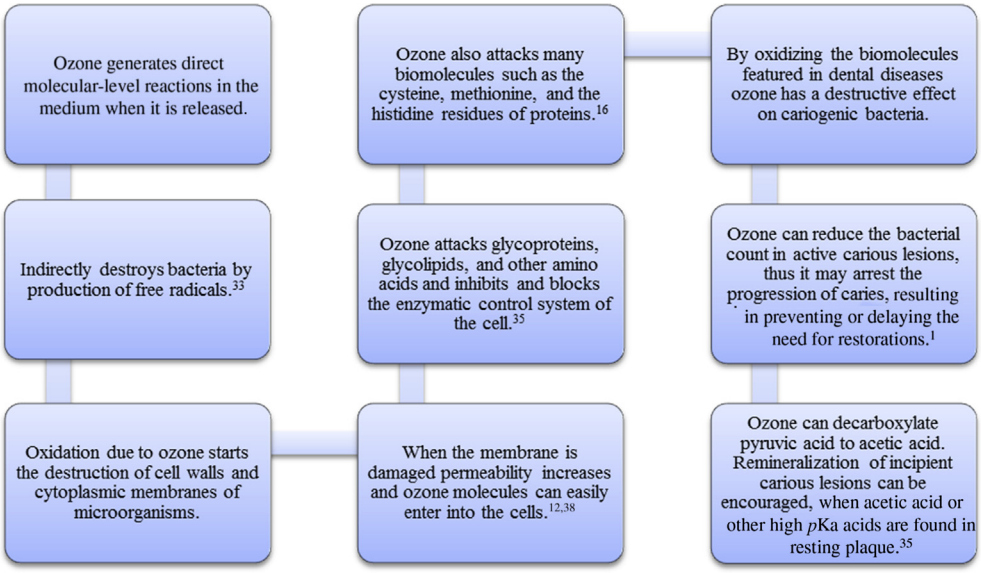

Figure 1 The mechanism of ozone's action.

Dähnhardt et al[17] have evaluated the efficacy of ozone gas on treating open carious lesions in anxious children with a prospective controlled clinical study. Two lesions in each child were treated with either ozone gas for 20 seconds or left untreated as the control group. The changes for hardness and DIAGNOdent values were compared for up to 8 months. Statistically significant improvement was observed between baseline and follow-up in the ozone-treated group. Moreover, it was reported that the level of fear reduced prior to the second session and following the last session.

Baysan and Lynch[45] assessed the safety and efficacy of ozone either with or without a root sealant for the management of leathery root caries. They separated the groups as follows: ozone only group, ozone with a root sealant, root sealant only, and the control group with no treatment. At the 6-month recall, 38.1% of lesions became hard in the ozone only group, whereas none of the lesions became hard in the control group. The ozone and sealant group also had greater improvements in the Electronic Caries Monitor and DIAGNOdent values when compared to the sealant only group. It is reported that ozone with or without a sealant could be safety and efficient therapy for the management of leathery root caries.

In a clinical study, the effect of ozone compared with Cervitec/Fluor Protector was assessed on avoiding demineralization around brackets. Patients with a poor oral hygiene who required multibracket appliance therapy were treated with ozone or a combination of Cervitec and Fluor Protector. The visible plaque index and white spot formation were analyzed clinically. The preventive effect of Cervitec/Fluor Protector on the development of white spot lesions during multibracket therapy was found to be superior to ozone.[46]

Hauser-Gerspach et al[47] compared the effectiveness of gaseous ozone as a disinfectant and chlorhexidine gel in the reduction of microorganisms in occlusal caries lesions of small children with and without excavation. Standardized samples were taken from each lesion prior to and after ozone or chlorhexidine application and microbiological counts were compared. It was reported that ozone application as well as 1% chlorhexidine gel application for 30 seconds was not effective in reducing microorganisms in open occlusal carious lesions. The removal of decayed tissue had no effect either.

In an *in vivo* setting, the efficiency of ozone alone and with a remineralizing solution following application on initial pit and fissure caries lesions in permanent molars was evaluated. After ozone treatment (40 s), patients either using the remineralizing solution or not were followed up at 1 month, 2 months, 3 months, and 6 months. The results have shown that using the remineralizing solution had no additional effect on the remineralization of initial pit and fissure caries lesions treated with ozone. The authors concluded that ozone treatment either alone or combined with a remineralizing solution was found to be effective for the remineralization of initial fissure caries lesions.[48]

Most of the clinical studies reported that ozone is a promising alternative to conventional methods for the management of caries. However, a few studies have shown ozone to be insufficient for preventing caries and reducing microorganisms in open occlusal carious lesions.[46,47] Table 1 shows a summary of these studies.

In vitro studies

There are several studies evaluating antimicrobial effect of ozone on cariogenic bacteria. Baysan et al[39] have evaluated the antimicrobial effect of ozone on primary root carious lesions and the efficacy on *Streptococcus mutans* and *Streptococcus sobrinus*. A significant reduction in microorganisms was observed after ozone exposure for either 10 seconds or 20 seconds in primary root carious lesions; furthermore, the application of ozone for 10 seconds was able to reduce the count of *S. mutans* and *S. sobrinus* on saliva-coated glass beads. The authors concluded that ozone may be an effective alternative to traditional drilling and filling for the treatment of primary root caries.

One of the preventive strategies of caries is reducing the amount of caries associated bacterial species in the dental plaque.[6] Nagayoshi et al's[19] *in vitro* study examined the effect of ozonated water on oral microorganisms and dental plaque. Ozonated water strongly inhibited the accumulation of experimental dental plaque and it was highly effective in killing both gram-positive and gram-negative oral microorganisms, almost no microorganisms were detected after being treated with ozonated water (4 mg/L) for 10 seconds.

Residual bacteria may cause secondary caries under restorations; therefore antimicrobial treatment could be useful. The antibacterial effect of ozone has been evaluated on *S. mutans* and compared with two antibacterial dentin bonding systems in a previous study. The authors reported that an 80 second application of ozone is a very promising therapy for eliminating residual microorganisms in deep cavities and therefore has the potential to increase the clinical success of restorations. The antimicrobial effect of bonding systems and 80 seconds of ozone were found to be significantly higher than that of the 40 second ozone treatment. A longer period of ozone activity could be advantageous as a result of its anticariogenic effect.[18]

The ability of ozone to kill microorganisms associated with noncavitated occlusal caries was investigated by Baysan and Beighton[20] They determined the effect of ozone on the viability of bacteria in the exposed infected dentine associated with these lesions. Forty-second ozone treatment of the lesions failed to significantly reduce the count of viable bacteria in infected dentin under demineralized enamel. The results of this study were contradictory to other studies' results.

Müller et al[3] have assessed the antimicrobial potential of ozone gas and photodynamic therapy on oral biofilm. Their results suggest that well-established biofilms are resistant to ozone application. Both ozone and photodynamic therapy had a minimal effect on the viability of microorganisms organized in a cariogenic biofilm. The authors concluded that ozone and photodynamic therapy are not currently a valid option to be eligible as a supportive measure in cases where mechanical removal of bacteria is not possible such as fissures, around orthodontic brackets, or in residual caries.

In a previous study, the antimicrobial effect of 0.1-ppm ozonated water was analyzed on 24-hour supragingival plaque microorganisms. The microbiological tests performed in the study suggested that ozonated water exhibited some antimicrobial activity on the bacterial

Table 1 Ozone in clinical studies.

Refs	Objective	Application time and form of ozone	Concentration of ozone	Results/conclusions
Holmes, 2003[16]	To assess the effect of an ozone delivery system, combined with the daily use of a remineralizing patient kit, on the clinical severity of noncavitated leathery PRCLs, in an older population group	40 s Gaseous ozone	2,100 ± 200 ppm with a flow of 615 mL/min	At 18 mo, 100% reversal and remineralization had been achieved. Leathery noncavitated primary root caries could be arrested nonoperatively with ozone and remineralizing products
Baysan and Lynch, 2004[43]	To assess the effect of ozone on the microbial flora and clinical severity of primary root caries	10 s and 20 s Gaseous ozone	2,100 ± 200 ppm with a flow of 615 mL/min	Ozone application for either 10 or 20 s dramatically reduced most of the microorganisms in primary root caries lesions without any side effects
Huth et al, 2005[44]	To investigate the effect of ozone on noncavitated initial occlusal fissure caries lesions with a split mouth design considering the patient's current caries risk	40 s Gaseous ozone	2,100 ± 200 ppm with a flow of 615 mL/min	The ozone-treated lesions showed significantly more caries reversal or reduced caries progression than the untreated control lesions within the group of patients at high current caries risk
Dähnhardt et al, 2006[17]	To evaluate the efficacy of ozone gas on treating open carious lesions in anxious children	20 s Gaseous ozone	2,100 ± 200 ppm with a flow of 615 mL/min	The use of ozone resulted in an average reduction of 13% of the laser fluorescence values immediately after the ozone treatment
Baysan and Lynch, 2007[45]	To assess the safety and efficacy of ozone either with or without a root sealant for the management of leathery root caries	10 s Gaseous ozone	2,100 ± 200 ppm with a flow of 615 mL/min	The ozone + sealant group and the ozone only group showed improvements in the Electronic Caries Monitor and DIAGNOdent values. Ozone was found to be efficient for the management of leathery root caries
Kronenberg et al, 2009[46]	To evaluate the effect of ozone comparing with Cervitec/Fluor Protector on avoiding demineralization around brackets	30 s Gaseous ozone	2,100 ± 200 ppm with a flow of 615 mL/min.	Preventive effect of Cervitec/Fluor Protector on the development of white spot lesions during multibracket therapy has been found to be superior to ozone.
Hauser-Gerspach et al, 2009[47]	To compare the immediate effects of gaseous ozone and chlorhexidine gel on bacteria in cavitated carious lesions in children	30 s Gaseous ozone	2,100 ± 200 ppm with a flow of 615 mL/min.	Ozone application as well as 1% chlorhexidine gel application for 30 s was not effective in reducing microorganisms in open occlusal carious lesions.
Atabek and Oztas, 2011[48]	To evaluate the efficiency of ozone alone and with a remineralizing solution following application on initial pit and fissure caries lesions in permanent molars	40 s Gaseous ozone	2,100 ± 200 ppm with a flow of 615 mL/min.	Ozone treatment either alone or combined with a remineralizing solution was found to be effective for remineralization of initial fissure caries lesions.

PRCL = primary root carious lesions.

Table 2 Ozone in *in vitro* studies.

Refs	Objective	Application time and form of ozone	Concentration of ozone	Results/conclusions
Baysan et al, 2000[39]	To evaluate the antimicrobial effect of ozone on primary root carious lesions and the efficacy on Streptococcus mutans and *Streptococcus sobrinus*	10 s and 20 s Aqueous ozone	0.069 mL and 0.0138 mL	A significant reduction in microorganisms was observed after ozone exposure for either 10 or 20 s in primary root carious lesions; moreover, the application of ozone for 10 s was able to reduce the count of *S. mutans* and *S. sobrinus* on saliva-coated glass beads
Nagayoshi et al, 2004[19]	To examine the effect of ozonated water on oral microorganisms and dental plaque	10 s, 30 s, 60 s, and 120 s Aqueous ozone	0.5 mg/L, 2 mg/L, and 4 mg/L	Ozonated water strongly inhibited the accumulation of experimental dental plaque, and it was highly effective in killing both gram-positive and gram-negative oral microorganisms; almost no microorganisms were detected after being treated with ozonated water (4 mg/L) for 10 s.
Polydorou et al, 2006[18]	To evaluate the antibacterial effect of ozone on *S. mutans* and compare with two antibacterial dentin bonding systems	40 s and 80 s Gaseous ozone	2,100 $\pm$ 200 ppm with a flow of 615 mL/min.	All treatments reduced the number of *S. mutans*. The antimicrobial effect of bonding systems and 80 s of ozone were found significantly higher than the 40-s ozone treatment.
Baysan and Beighton, 2007[20]	To investigate the ability of ozone to kill microorganisms associated with noncavitated occlusal caries	40 s Gaseous ozone	2,100 $\pm$ 200 ppm with a flow of 615 mL/min.	Ozone treatment of the lesions failed to significantly reduce the count of viable bacteria in infected dentin under demineralized enamel.
Müller et al, 2007[3]	To assess antimicrobial potential of ozone gas and photodynamic therapy on oral biofilm	60 s Gaseous ozone	2,100 $\pm$ 200 ppm with a flow of 615 mL/min.	Both ozone and photodynamic therapy had a minimal effect on the viability of microorganisms organized in a cariogenic biofilm.
Sadatullah et al, 2012[49]	To analyze the antimicrobial effect of ozonated water on 24-hour supragingival plaque microorganisms	30 s Aqueous ozone	0.08-0.1 ppm	Exposure to the ozonated water for 30 s reduced the total bacteria population of the 24-hour plaque by 45.3%.
Zaura et al, 2007[50]	To test the hypothesis that ozone promotes remineralization of dentinal lesions *in vitro*	60 s Gaseous ozone	2,100 $\pm$ 200 ppm with a flow of 615 mL/min.	The exposure of ozone had no effect on remineralization and subsequent demineralization of remineralized dentinal lesions.

(*continued on next page*)

Table 2 (*continued*)

Refs	Objective	Application time and form of ozone	Concentration of ozone	Results/conclusions
Bezirtzoglou et al, 2008[51]	To estimate the ozone experimental effect upon toothbrushes microflora microbiologically prior to and after saturation with ozone gas	5 min, 10 min, 15 min, 20 min, and 30 min Aqueous ozone	3-3,5 ppm 100 μL	Ozone application removed the toothbrushes bristles microbiota and maximum decontamination efficacy of ozone treatment was observed after 30 min.
Castillo et al, 2008[33]	To evaluate the antimicrobial effect of ozone gas on mutans streptococci	10 s, 20 s, and 40 s Gaseous ozone	2,100 ± 200 ppm with a flow of 615 mL/min.	Ozone application for 10 s and 20 s produced a significant reduction in the number of bacteria. When exposure lasted 40 s, no viable bacteria were obtained.
Knight et al, 2008[52]	To determine the effects of prior ozone application to dentine on biofilm formation and to measure any associated reduction in bacteria viability	40 s Gaseous ozone	2,100 ± 200 ppm with a flow of 615 mL/min.	The infusion of ozone into noncarious dentine prevented biofilm formation in vitro from *S. mutans* and *Lactobacillus acidophilus* over a 4-wk period
Johansson et al, 2009[7]	To evaluate the antibacterial effect of ozone on cariogenic bacterial species with and without the presence of saliva	10 s, 30 s, and 60 s Gaseous ozone	2,100 ± 200 ppm with a flow of 615 mL/min.	The tested cariogenic species were affected to various degrees by ozone gas with almost 100% killing after only 60 s of ozone application. The presence of saliva reduced the antibacterial effect of ozone.
Polydorou et al, 2012[5]	To evaluate the antimicrobial effect of ozone application on *S. mutans* and *Lactobacillus casei*, 4 wks and 8 wks after the treatment, using a tooth cavity model	60 s Gaseous ozone	2,100 ± 200 ppm with a flow of 615 mL/min.	The ozone application resulted in the reduction of *S. mutans* after the first 4 wks, even after 8 wks. However *L. casei* was found to be more resistant to ozone.

population of the 24-hour plaque. Exposure to the ozonated water for 30 seconds reduced the total bacteria population of the 24-hour plaque by 45.3%. The study suggested that ozonated water rinsing may be an extremely useful addition to tooth brushing and flossing.[49]

Zaura et al[50] tested the hypothesis that ozone promotes the remineralization of dentinal lesions *in vitro*. Artificial caries-like lesions in dentin were treated with ozone gas, the samples were remineralized and subsequent demineralized again and mineral content was assessed by transverse microradiography. According to the results, the authors concluded that the exposure of ozone had no effect on the remineralization and subsequent demineralization of remineralized dentinal lesions.

The ozone experimental effect upon toothbrushes microflora was estimated microbiologically prior to and after saturation with ozone gas by Bezirtzoglou et al.[51] They reported that ozone application removed the toothbrushes bristles microbiota following conventional brushing, and maximum decontamination efficacy of ozone treatment was observed after 30 minutes, whereas exposure for short time periods (5 minutes, 10 minutes, 15 minutes, 20 minutes) seems to be inefficient, which probably reflects the low dose of ozone used in the study.

Castillo et al[33] have evaluated the antimicrobial effect of ozone gas on mutans streptococci. Their results showed that ozone application for 10 seconds and 20 seconds produced a significant reduction in the number of bacteria; when exposure lasted for 40 seconds, total elimination of bacteria was observed. Both the time of application and the previous bacterial concentration influence the antimicrobial effect of ozone.

The treatment of dentine surfaces to prevent biofilm formation and reduce bacteria growth may assist in the prevention of caries initiation and progression. In an *in vitro* study, the effects of prior ozone application (40 seconds) to dentine on biofilm formation was determined and the reduction in bacteria viability was measured. The results of the study has shown that the infusion of ozone into noncarious dentine prevented biofilm formation *in vitro* from *S. mutans* and *Lactobacillus acidophilus* over a 4-week period.[52]

Johansson et al[7] evaluated the antibacterial effect of ozone on cariogenic bacterial species with and without the presence of saliva and a possible effect on the salivary proteins. The study showed that the tested cariogenic species are affected to various degrees by ozone gas with almost 100% killing after only 60 seconds of ozone application. It was also shown that the presence of saliva reduced the antibacterial effect of ozone although increasing the ozone application time to 60 seconds overcame these reductants. The detection of altered salivary proteins indicated that salivary components are additional targets for ozone molecules.

Recently, Polydorou et al[5] evaluated the antimicrobial effect of ozone application on the two most important cariogenic species, *S. mutans* and *Lactobacillus casei*, 4 weeks and 8 weeks after the treatment, using a tooth cavity model. The results indicated that the effect of ozone differed between the two types of microorganisms. It was shown that the viability of *L. casei* was not affected by the ozone application; by contrast, the viability *S. Mutans* reduced significantly. The ozone application resulted in a reduction of bacteria after the first 4 weeks. As far as its effect through the time period of 8 weeks is concerned, it seemed to be stable through time. It is suggested that application of ozone in addition to the use of other antibacterial methods after caries excavation might be more successful in order to eliminate the remaining bacteria under the restorations.

Ozone might be a useful tool to reduce and control oral infectious microorganisms in dental plaque and dental cavity.[35] However, the results of *in vitro* studies are controversial, although some researchers reported that ozone therapy had a minimal or no effect on the viability of microorganisms,[3,20] others suggested ozone to be highly effective in killing both gram-positive and gram-negative oral microorganisms (Table 2).[19,33,49,51]

Adverse effects, safety, and cytotoxicity of ozone

As there is limited evidence of the benefits of gaseous ozone in its application in dentistry, current National Institute of Clinical Excellence (NICE) guidelines[53] advises against using ozone alone in the treatment of caries in general dental practice except as part of an approved clinical trial. On the basis of these terms, Millar and Hodson[54] have evaluated the safety of two ozone delivery devices designed for use in dentistry. Ozi-cure and HealOzone devices were used in a clinical simulation using a phantom head, whereas recordings of ozone levels were made in the pharyngeal and nasal regions of the patient and near the mouth of the operator. According to the results, the authors concluded that the Ozi-cure device when used without adequate suction allows ozone to reach a concentration above permitted levels and therefore should not be used. The HealOzone device was safe to use.[54]

A previous study investigated whether gaseous and aqueous ozone exert any cytotoxic effects on human oral epithelial (BHY) cells and gingival fibroblast (HGF-1) cells compared with different antiseptics. Cell counts, metabolic activity, Sp-1 binding, actin levels, and apoptosis were evaluated. Ozone gas was found to have toxic effects on both cell types. Essentially no cytotoxic signs were observed for aqueous ozone. Aqueous ozone revealed the highest level of biocompatibility of the tested antiseptics.[42]

In the literature, few clinical studies have shown that no adverse effects were found with ozone therapy[43,45]; therefore, more studies are needed to support its safety.

Conclusion

In many *in vivo* and *in vitro* studies, ozone has been used for the treatment of caries, disinfection of the cavity, reducing the levels of caries-associated microorganisms in the dental plaque, and remineralization of caries lesions with successful results. However, the clinical evidence for application of ozone is not extensive. Therefore more evidence is required before ozone can be accepted as an alternative to present methods for the management and prevention of caries.

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