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Ozone and Caries: A Review of the Literature

Abstract: Ozone, either in gaseous form or as ozonated water, has been available for use as a treatment for dental caries for a decade. This paper reviews the literature on the subject by examining the findings of publications in the peer review literature. Eighteen papers were identified by a literature search. From the review of these, it was concluded that, while some laboratory studies and some short duration clinical studies have suggested that ozone may be effective in the treatment of root caries or killing of oral micro-organisms, the clinical evidence for the use of ozone in treatment of caries is not compelling.

Clinical Relevance: It may be considered that there is presently no justification for the routine use of ozone in the treatment of caries.

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Ozone: what is known

Ozone, first discovered in 1840, is a naturally occurring compound consisting of three oxygen atoms. It is found in nature in the form of a gas in the atmosphere in a concentration of 1–10 ppm¹ where it has a role in the thermal structure of the stratosphere. It is well known as a disinfectant, powerful oxidant and antimicrobial agent, having been used in World War I for treating gas gangrene, infected wounds and the like. It is a strong oxidizer of cell walls and the cytoplasmic membranes of bacteria, making it a bactericidal, antiviral and antifungal agent.² However, it has been considered to lack the ability to discriminate between various targets in the environment and bacteria, potentially affecting the outcome of antibacterial treatment.³

Today, ozone is a useful disinfectant used in a variety of areas, including:

- The food industry, for example in food

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Figure 1. The *Healozone* ozone generating device (courtesy of Dr Mark Hampson, Yeovil, UK).

sterilization/shelf-life extension, prevention of growth of mould during storage of fruit and in equipment sterilization;

- The chemical industry, for example, for producing peroxides and for rapid drying of varnishes and inks; and
- It has also been used as a disinfecting

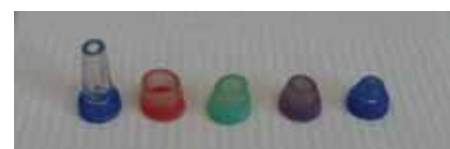


Figure 2. A variety of suction tips utilized with *Healozone* (courtesy of Dr Mark Hampson, Yeovil, UK).



Figure 3. *Healozone* in use (courtesy of Dr Mark Hampson, Yeovil, UK).

agent in drinking water, in swimming pools, in sewage treatment, air and as a bactericide.

The use of ozone in surgery was reported to the German Surgical Society in 1935.⁴ Meanwhile interest in the use of ozone in dentistry expanded dramatically with the introduction of the KaVo *Healozone* (Curozone: KaVo Biberach/Riss, Germany) ozone generating system which delivers ozone gas at a concentration of 2,100 ppm +/-10%, via a sealing silicone cup of 3–8 mm diameter applied to the tooth surface (Figures 1 and 2). The application procedure (Figure 3) normally

Author(s)	Year of publication	Number of teeth/specimens in study	Ozone successful [✓] or unsuccessful [X]	Comments
Holmes ⁸	2003	RCCT, split mouth: 57 pairs of initial fissure caries lesions treated, half with ozone, monitored using <i>Diagnodent</i>	✓	100% of the ozone-treated PRCLs had arrested at 18 months
Baysan A <i>et al</i> ⁷	2000	Biopsy with sterile excavator from PRCL. 50% treated with ozone, recall 3–5.5 months	✓	10 seconds ozone application
Huth <i>et al</i> ¹⁰	2005	Split mouth single blind RCT design. Ozone vs untreated controls. Lesions followed for 3 months.	✓	Caries progression monitored by <i>Diagnodent</i>
Dahnhardt <i>et al</i> ¹⁵	2006	82 open Class I caries lesions in unco-operative children treated with ozone, followed for 8 months	✓	Hardness values and laser fluorescence of treated lesions improved
Kronenberg <i>et al</i> ¹⁶	2009	Split mouth design: 20 patients with poor OH with orthodontic brackets, treated with ozone or chlorhexidine/thymol varnish and fluoride varnish	X	The caries protective effect of the varnish was superior to that of ozone
Hauser-Gerspach <i>et al</i> ¹⁷	2009	Open occlusal caries lesions in children treated with ozone. Total reduction in cultivable anaerobes was 7% and 19% in excavation/non-excitation groups	X	Ozone application (as well as 1% chlorhexidine gel application) had no effect in reducing micro-organisms in open occlusal caries lesion. Removal of decayed tissue had no effect

Table 1. Papers on the effect of ozone *in vivo*.

takes between 20 and 120 seconds per tooth. After the ozone application, the tooth surface is treated with a remineralizing solution containing fluoride, calcium, zinc, phosphate and xylitol dispensed from a 2 ml ampoule. Patients are also supplied with a patient kit which contains toothpaste, oral rinse and oral spray, all containing fluoride, calcium, zinc, phosphate and xylitol.⁵ It is suggested that the *Healozone* application is repeated at 3 months and 6 months.

Methods

A Medline search was carried out in 2009 using the key words Ozone and Caries. For the purpose of this review, laboratory and clinical studies were included. When the

same study was identified in more than one clinical publication, the publication giving details of the longest period of evaluation was used. Only papers from peer-reviewed publications were included, as it was considered that the peer review process would help produce data of the highest validity. Research abstracts were not included, as these do not include the full methodological details which may be considered necessary to inform a judgement on whether their inclusion is appropriate. Additionally, research of good quality, while it may appear initially as an abstract, should become available in the peer-reviewed literature in due course. This paper is intended to complement two systematic reviews on the subject which

were published in 2006⁵ and 2008.⁶ It will review the literature as identified from the search and include other literature identified from the references of relevant publications.

Results

Eighteen papers were identified by the search, with the results being summarized in Tables 1, 2 and 3. All used the ozone generator, *Healozone*, unless otherwise noted. The more significant findings, including the early studies which raised hope that a new form of treatment had arrived, are described in more detail below.

A chronological perspective

The earliest publication identified

Author(s)	Year of publication	<i>In vivo</i> or <i>in vitro</i>	Number of teeth/specimens in study	Ozone successful [✓] or unsuccessful [X]	Comments
Baysan <i>et al</i> ⁷	2000	<i>In vitro</i>	Primary root caries lesions (PRCL) in 40 extracted teeth	✓	
Baysan & Beighton ¹³	2007	<i>In vitro</i>	Non-cavitated occlusal surfaces of freshly extracted teeth treated with ozone and underlying dentine exposed	X	Ozone failed to reduce the number of micro-organisms in non-cavitated lesions
Muller <i>et al</i> ¹¹	2007	<i>In vitro</i>	Compared the reduction in colony forming units with photodynamic therapy (PDT) and gasiform ozone	X	Tested the effect on complex six-species biofilm
Johansson <i>et al</i> ³	2009	<i>In vitro</i>	Lab study on suspensions of Actinomyces, Lactobacilli, <i>S. mutans</i> exposed to ozone gas/Healozone	✓	Cariogenic species almost eliminated following 60s exposure to ozone
Fagrell <i>et al</i> ¹⁸	2008	<i>In vitro</i>	Studied effect of ozone on common oral micro-organisms	✓	Ozone treatment for 20s prevented bacterial growth
Zaura <i>et al</i> ¹⁹	2007	<i>In vitro</i>	Tested the hypothesis that ozone promotes remineralization of dentinal lesions <i>in vitro</i>	X	No difference between ozone and water groups
Polydorou <i>et al</i> ²⁰	2006	<i>In vitro</i>	Evaluated the antibacterial effect of Healozone on <i>S. mutans</i>	✓	80 second application showed 'striking antimicrobial effects'
Nagayoshi <i>et al</i> ²¹	2004	<i>In vitro</i>	Examined the effect of ozonated water on oral micro-organisms	✓	Ozonated water for 10s was effective for killing gram -ve and +ve organisms

Table 2. Papers on the effect of ozone *in vitro*.

which used ozone for treatment of caries was published in 2000.⁷ This examined the efficacy of ozone as an antibacterial agent on primary root caries lesions (PRCL) in 40 extracted teeth when exposed to ozonized water for 10s or 20s, and on saliva-coated glass beads covered with *Streptococcus mutans* and *Streptococcus sobrinus* with 10s exposure to ozone gas. Half of the PRCL was excavated using a sterile excavator, the remaining lesion being treated with the ozonized water. A significant reduction ($p < 0.001$) was observed in the ozone-treated groups. Similarly, the numbers of *S. mutans* and *S. sobrinus* were reduced. This publication raised hopes that a new form of

quick, conservative and easy treatment for caries had arrived as it confirmed previously considered views that ozonated water and ozone gas were effective in killing micro-organisms.

This was reinforced by a paper, in 2003, from Julian Holmes,⁸ a UK general dental practitioner. In a double-blind trial of PRCLs in 89 subjects (aged between 60 and 82 years) for 18 months, patients were treated with ozone or air, followed by remineralizing solution. This was repeated at 6, 12 and 18 months. A good 98% recall was achieved, bearing in mind the age of the subjects since, in studies involving subjects of similar age, the

recall rate could be expected to be reduced by deaths and serious illness. No details of the recall assessment and who carried it out were provided. The results indicated that 100% of the ozone-treated PRCLs had arrested at 18 months, while 37% of the control (not treated with ozone) lesions had gone from leathery to soft, a significant difference ($p < 0.01$).⁸ The author concluded that the ozone treatment regime was an effective alternative to conventional 'drilling and filling'.

In another study, ethical approval was obtained for treatment of 70 PRCLs in 26 patients.⁹ A 'biopsy' was taken from one half of the lesion using a sterile excavator,

Author(s)	Year of publication	<i>In vivo</i> or <i>in vitro</i>	Ozone successful [✓] or unsuccessful [X]
Cochrane/ Rickard <i>et al</i> ²²	2004	<i>In vivo</i>	X
NICE ²³	2005	<i>In vivo</i>	X
Brazzelli <i>et al</i> ⁵	2006	<i>In vivo</i>	X
Azarpazoooh & Limeback ⁶	2008	<i>In vivo</i>	X

Table 3. Systematic reviews on the use of ozone in treatment of caries.

and this was sent for microbiological analysis: the other half of the lesion was treated with ozone, and again sent for microbiological analysis. A significant ($p < 0.001$) reduction in micro-organisms after ozone application was noted for either 10 or 20s applications, with the authors concluding that: 'Ozone application for 10 or 20 seconds dramatically reduced most of the micro-organisms in PRCLs with no side-effects at recalls of up to 5.5 months and that the technique might be considered a potentially effective alternative to conventional 'drilling and filling'.⁹

Another study, published in 2005 by a team from the University of Munich,¹⁰ investigated the effect of ozone on caries lesions in first permanent molar teeth in children in a randomized controlled clinical study. The results, albeit only at 3 months, and using *Diagnodent* to monitor the lesions, indicated that ozone-treated lesions successfully showed more caries reversal or reduced caries progression than the untreated lesions.¹⁰ On the other hand, results of a laboratory study by Muller and colleagues indicated that, when tested on the more realistic multispecies oral biofilm, ozone therapy was not successful.¹¹ However, closer examination of the paper suggests that the researchers did not follow the manufacturer's directions in the use of the apparatus.

A review paper by Baysan and Lynch,¹² in 2006, demonstrated the safety of the *Healozone* machine and concluded that the 'use of ozone in the treatment of root caries looks very promising'. The authors added that 'there is a need for further research'. However, these authors do not quote the results of the Holmes paper, which was published earlier (in 2003), despite it

involving the use of ozone in the clinical treatment of root caries.

Further elucidation of the process by which ozone may affect the caries process was given by Baysan and Beighton in 2007.¹³ These workers carried out microbiological sampling of 104 freshly extracted (15 minutes following extraction) molar teeth which had non-cavitated, occlusal caries lesions, half of which were treated with ozone gas. The enamel was then removed to allow bacterial sampling, with the results indicating that there was no significant difference between the number of bacteria recovered from the dentine of ozone treated and non-treated groups. The authors discussed these findings in the light of previous work and considered that the concentration of ozone in the aqueous phase was insufficient to exert an antimicrobial effect.¹³ Lynch, however, in a reply to a review paper by Hodson and Dunne which quoted the above work, pointed out that the manufacturer's recommendations for the use of *Healozone* had not been carried out.¹⁴

Other *in vivo* and *in vitro* studies

A summary of the results from the review are presented in Tables 1 and 2. Four of the six *in vivo* papers indicate an effect of ozone on cervical caries. Five of the eight *in vitro* studies demonstrated a positive effect on cariogenic bacteria.

Systematic reviews

Systematic reviews may be considered the highest form of 'evidence', with the exception of meta-analyses, so their results, in synthesizing the data on a given

topic, must be held in the highest regard.

The first systematic review on ozone in the treatment of caries, a Cochrane Review, was carried out by Rickard and colleagues and published in 2004.²² Inclusion criteria were:

- Randomization in a controlled trial;
- Ozone application vs no application in control group;
- Outcomes measured for at least 6 months.

Two published studies, a large number of conference abstracts, two PhD theses and a submitted manuscript were located during the search or provided by authors known to be researching the subject. The authors excluded 42 conference papers, abstracts and posters (from an unknown number of studies) and included three trials with a total of 432 randomized lesions. The review concluded that: 'Given the high risk of bias in the available studies and the lack of consistency between different outcome measures, there is no reliable evidence that application of ozone gas to the surface of decayed teeth stops or reverses the decay process. There is a fundamental need for more evidence of appropriate rigour and quality before the use of ozone can be accepted into mainstream primary dental care'. In addition, the authors added that 'Dental care providers should not advocate the use of ozone on the sole basis of high patient acceptability as compared with less attractive drilling and filling'.

The second systematic review was carried out by the UK's National Institute for Health and Clinical Excellence (NICE),²³ concluding as follows: 'NICE has concluded that there is not enough reliable evidence to show that *Healozone* is more effective than existing treatments for decay of the biting surfaces and roots of the teeth. The authors added that they 'will consider a review of the guidance in 2008 when we hope there will be further good quality evidence available at that time'.

At the time of writing this paper, no such review has been published.

The third review was published by Brazzelli and colleagues.⁵ Inclusion criteria were:

- RCTs of ozone treatment vs at least one comparator;
- Minimum 6 month follow-up.

Five full text reports on the use of *Healozone* in management of occlusal caries and five studies published as abstracts

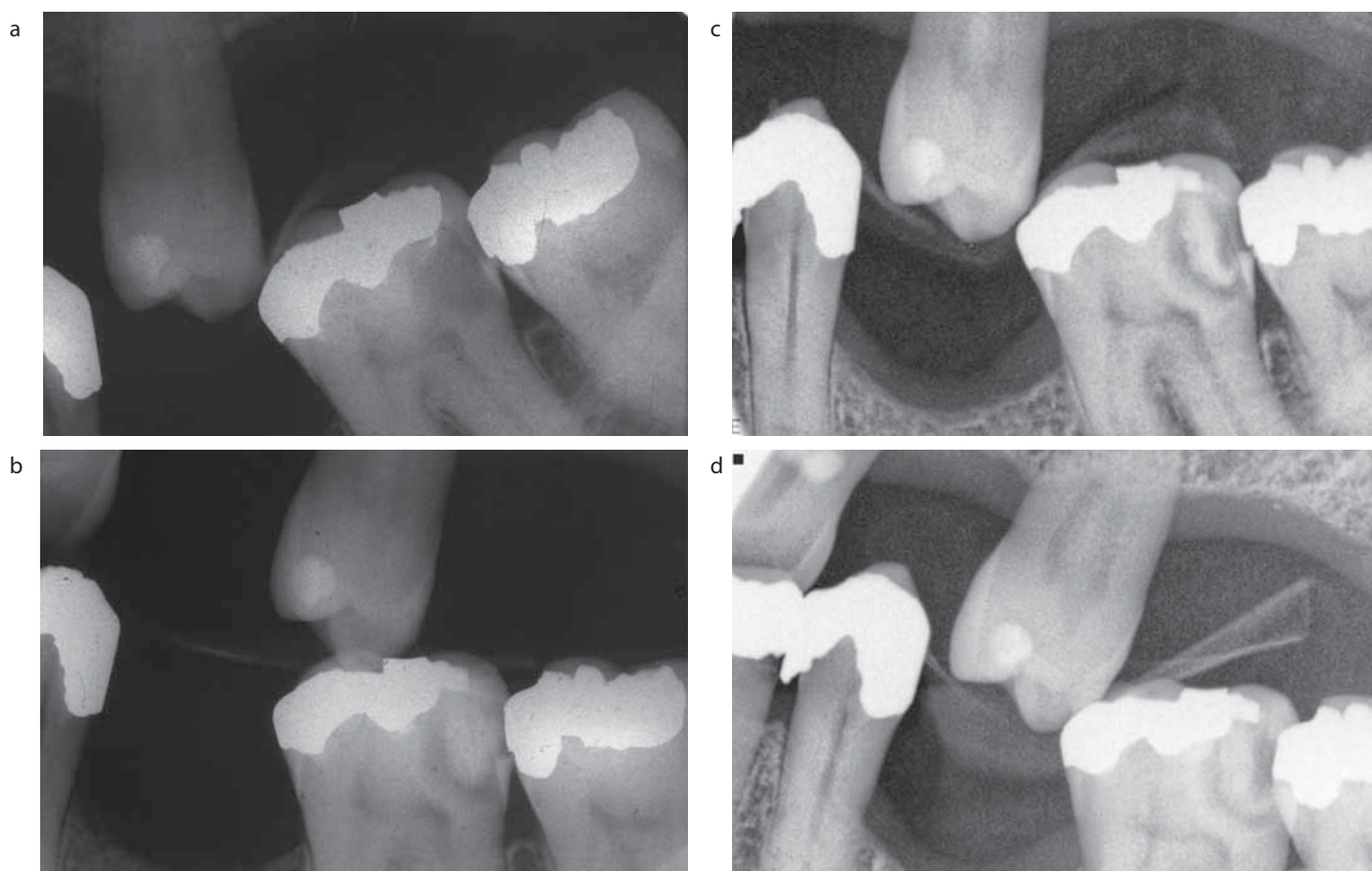


Figure 4. (a–d) Progressive remineralization of a deep caries lesion treated with *Healozone* (courtesy of Dr Mark Hampson, Yeovil, UK).

(four on occlusal caries one on root caries) met the inclusion criteria. In the discussion in the paper, the authors stated that: 'Nearly all the research to date comes from the same group who developed and pioneered the procedure... and that there is a need for trials to be carried out by independent research teams: they should be properly randomized, apply appropriate statistical methods, use validated and reproducible criteria for the assessment of caries, mask participants and outcome assessors, should measure caries incidence for at least two years, but realistically much longer.' They concluded that 'any treatment that preserves teeth and avoids fillings is welcome. However, the current evidence base for *Healozone* is insufficient to conclude that it is a cost-effective addition to the management and treatment of occlusal and root caries.'

The most recent systematic review, published in 2008,⁶ was carried out by Azarpazooch and Limeback. It concluded, on *in vitro* results, that there was good evidence

of ozone biocompatibility with human oral epithelial cells but conflicting evidence of the antimicrobial efficacy of ozone. On the *in vivo* data, the authors concluded that 'despite the promising *in vitro* evidence, the clinical application of ozone in management of dental and root caries has not achieved a strong level of efficacy and cost-effectiveness', concluding that 'the promising potential of ozone has not been realized in clinical studies to date'. Lynch²⁴ criticized the review by Azarpazooch and Limeback for having criticized subjective tactile hardness for the clinical severity score as being a methodological concern. He stated that hardness was the most reliable clinical measure of lesion severity but did not reference this statement. Azarpazooch and Limeback²⁵ were given the last word in this discussion, stating: 'We are happy that Dr Lynch, as one of the experts in the field, has found many useful parts in the paper'. However, they stated that:

■ There is no consensus on the applied dose of ozone;

■ Overall, the *in vitro* studies show a promising potential for ozone in dentistry;

■ We criticize Dr Lynch's conclusion that 'Ozone's place is to use its proven antimicrobial efficacy to reduce cariogenic micro-organisms. The results of an *in vitro* study would not be generalizable to clinical practice';

■ We should remain evidence-based in our practice and have conversations with our patients.

In summary, Table 3 demonstrates that four systematic reviews failed to ascertain the cariostatic effect of ozone.

Safety

Millar and Hodson²⁶ tested the safety of ozone devices using a phantom head clinical simulation while ozone levels were monitored in the pharyngeal and nasal regions of the 'patient'. The results indicated that *Healozone* was safe to use, but that the *Ozi-cure* device should not be used without adequate suction, as inadequate suction

allowed the ozone levels to reach a concentration above permitted levels. The *Ozi-cure* device has now been superseded by *Ozo-top* (Tip Top Tips Sarl, Rolle, Switzerland).

Finally, the author has received a variety of reports, from UK-based general dental practitioners, of clinical reversal of caries lesions, with one such report being presented as Figures 4 (a–d). While reports such as this are helpful, it could be considered appropriate that a long-term, randomized, clinical controlled trial should be carried out to demonstrate, once and for all, whether the exponents of ozone treatment of caries are correct in their beliefs, or whether the procedure should be confined to the annals of history.

Conclusion

This review has concluded that, while some laboratory studies and some short duration clinical studies have suggested that ozone, either in the form of gas, or as ozonated water, may be effective in the treatment of root caries or killing of oral micro-organisms, the clinical evidence from systematic reviews for the use of ozone in the treatment of caries is not yet compelling. There remains a need for one or more well-designed, randomized, clinical controlled trials to be carried out.

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