Review

The application of ozone in dentistry: A systematic review of literature

Amir Azarpazhooh, Hardy Limeback

Department of Endodontics and Community Dental Health Services Research Unit, Faculty of Dentistry, University of Toronto, Room #521A, 124 Edward Street, Toronto, ON, M5G 1G6 Canada
Department of Preventive Dentistry, Faculty of Dentistry, University of Toronto, 124 Edward Street, Toronto, ON, M5G 1G6 Canada

Objectives: (1) To systematically review the clinical application and remineralization potentials of ozone in dentistry; (2) To summarize the available in vitro applications of ozone in dentistry.

Sources: Ovid MEDLINE, CINAHL, etc. (up to April 2007).

Study selection: In vitro or in vivo English language publications, original studies, and reviews were included. Conference papers, abstracts, and posters were excluded.

Results: In vitro:

- Good evidence of ozone biocompatibility with human oral epithelial cells, gingival fibroblast, and periodontal cells;
- Conflicting evidence of antimicrobial efficacy of ozone but some evidence that ozone is effective in removing the microorganisms from dental unit water lines, the oral cavity, and dentures;
- Conflicting evidence for the application of ozone in endodontics;
- Insufficient evidence for the application of ozone in oral surgery and implantology;
- Good evidence of the prophylactic application of ozone in restorative dentistry prior to etching and the placement of dental sealants and restorations.

In vivo: Despite the promising in vitro evidence, the clinical application of ozone in dentistry (so far in management of dental and root caries) has not achieved a strong level of efficacy and cost-effectiveness.

Conclusions: While laboratory studies suggest a promising potential of ozone in dentistry, this has not been fully realised in clinical studies to date. More well designed and conducted double-blind randomised clinical trials with adequate sample size, limited or no loss to follow up, and carefully standardised methods of measurement and analyses are needed to evaluate the possible use of ozone as a treatment modality in dentistry.

© 2007 Elsevier Ltd. All rights reserved.
1. **Introduction**

Ozone (also known as triatomic oxygen and trioxygen) is a naturally occurring compound consisting of three oxygen atoms. It is found in nature, in the form of a gas in the stratosphere in a concentration of 1–10 ppm, being continually created from and destroyed into molecular O\(_3\). Both these chemical reactions are catalyzed by very high frequency ultraviolet light from sunlight. Consequently harmful B and C ultraviolet radiations in the stratosphere reaching the outer atmosphere from the sun are absorbed by ozone. Therefore, ozone in stratosphere has a critical role in both the thermal framework for life on the Earth’s surface. On the other hand, ozone in the troposphere is considered to be toxic for the pulmonary tract. In the troposphere, ozone is produced in a complicated series of chemical reactions involving the components of automobile exhaust (NO\(_3\)), sunlight (especially in hot summer months), and oxygen.

The reliable microbiologic and metabolic properties of ozone, in either the gaseous or aqueous phases, make it a useful disinfectant with a wide range of activity (Table 1). Ozone, in the gaseous or aqueous phase, has been shown to be a powerful and reliable antimicrobial agent against bacteria, fungi, protozoa, and viruses. It is generally accepted that the oxidant potential of ozone induces the destruction of cell walls and cytoplasmic membranes of bacteria and fungi. During this process, ozone attacks the cysteine, methionine, and the histidine residues of proteins. By oxidizing the biomolecules featured in dental diseases, ozone has a severely disruptive effect on cariogenic bacteria, resulting in elimination of acidogenic bacteria. The strongest naturally occurring acid, produced by acidogenic bacteria during cariogenesis is pyruvic acid. Ozone can decarboxylate this acid to acetic acid. It has been shown that remineralization of incipient carious lesions can be encouraged when the production of acetate acid, or other high pKa acids found in resting plaque, buffers plaque fluid.

During World War I, ozone gas was used for treating gaseous post-traumatic gangrene, infected wounds, mustard gas burns and fistulas in German soldiers. Ozone therapy was accepted as an alternative medicine in the USA from 1880 until 1932. To date, ozone therapy has been a recognized treatment modality in 16 countries. Its use has been investigated in treatment of ocular diseases (such as optic neuropathies, glaucoma, central retinal vein obstructions, and degenerative retinal diseases), acute and chronic bacterial, viral and fungal infections, ischemic diseases, age-related macular degeneration, orthopedic diseases, and dermatological, pulmonary, renal, hematological and neurodegenerative diseases. It can react with blood components (erythrocytes, leukocytes, platelets, endothelial cells and the vascular system) and positively affect oxygen metabolism, cell energy, the immunomodular property, antioxidant defence system, and microcirculation.

In dentistry, Dr. E.A. Fisch (1899–1966) was the first dentist to use ozonated water in his practice and introduced it to the German surgeon Dr. Erwin Payr (1871–1946) who used it from that time forward in surgery and reported his results at the 59th Congress of the German Surgical Society in Berlin (1935). In dental surgery, ozonated water was used to promote haemostasis, enhance local oxygen supply, and inhibit bacterial proliferation. Theoretically, ozone can reduce the bacterial count in active carious lesions and therefore, it may temporarily arrest the progression of caries, resulting in prevention or delaying the need for tooth restorations.

The primary objective of this article was to systematically review the clinical application and remineralization potentials of ozone in dentistry. More specifically, the report attempts to answer the following focused question: “How effective is ozone gas in prevention of dental caries?” Our secondary objective was to summarize the available in vitro studies in dentistry in which ozone has been used. This objective would be of importance to future researchers in terms of what has been tried and what the potentials are for the clinical application of ozone in dentistry.

### Table 1 – Major industrial uses of ozone

<table>
<thead>
<tr>
<th>Food industry</th>
<th>Chemical industry</th>
<th>Other industry uses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Food preservation</td>
<td>Oxidizing agent in the organic chemical industry</td>
<td>Disinfectant agent in drinking water and air</td>
</tr>
<tr>
<td>Shelf-life extension</td>
<td>Bleaching flour, paper pulp, starch, and sugar</td>
<td>Treating industrial wastes</td>
</tr>
<tr>
<td>Equipment sterilization</td>
<td>Processing certain perfumes, vanillin, and camphor</td>
<td>Deodorizing of feathers, air and sewage gases</td>
</tr>
<tr>
<td>Improvement of food plant effluent</td>
<td>Rapid drying of varnishes and printing inks</td>
<td>Bactericide</td>
</tr>
<tr>
<td>Disinfector agent of food in cold storage rooms</td>
<td>Producing peroxides</td>
<td>Producing steroid hormones</td>
</tr>
<tr>
<td>Food preservative for cold storage of meats</td>
<td>Removal of chlorine from nitric acid</td>
<td></td>
</tr>
<tr>
<td>Prevent the growth of yeast and mould in fruit storage</td>
<td>Oxidation of phenols and cyanides</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Aging liquor and wood</td>
<td></td>
</tr>
</tbody>
</table>

Source: Adopted from references [7] and [8].
2. Methods

2.1. Data sources

A comprehensive literature search of databases Ovid MEDLINE(R) and its allied versions, Cumulative Index to Nursing & Allied Health Literature, Evidence-Based Medicine of Cochrane Central Register of Controlled Trials, Cochrane Database of Systematic Reviews, Database of Abstracts of Reviews of Effects, EMBASE, Health and Psychosocial Instruments, HealthSTAR/Ovid Healthstar, International Pharmaceutical Abstracts, and internet resource of Google Scholar™ was carried out (from their inception until 31 April 2007) to identify studies where ozone was used. The websites of the manufacturing company (KaVo Dental GmbH, Germany and CurOzone USA Inc, Aurora, ON, Canada) were also searched for any other publication. Articles published in the English literature alone were reviewed. Table 2 shows the keywords and combinations of the keywords used.

2.2. Study selection

Randomised and quasi-randomised controlled trials of ozone application in dentistry were included. Retrospective studies, case series or case reports, letters to editors (not containing primary data), editorials, review articles, conference papers, abstracts, posters and commentaries were excluded but were read to identify potential studies.

2.3. Data extraction

After removing duplicates as well as reviewing the titles and abstracts of the retrieved articles, 45 articles were found to be relevant for our objectives. Reference lists of the retrieved articles were also searched to identify any other articles relevant to the research question, which may have provided additional information, resulting in identifying eight more studies.

3. Results

While the in vitro studies on this topic are important, the primary objective of this review was to find the evidence for the clinical application of ozone in dentistry. Therefore, the results of the search have been summarized in two sections:

(1) In vivo studies: a summary of available information with the reviewers’ evaluation and critical appraisal comments.
(2) In vitro studies: a summary of available information.

3.1. Primary objective: Clinical applications of ozone in dentistry

3.1.1. Ozone in the management of incipient caries

Three human in vivo studies were found to evaluate the efficacy of ozone in the management of incipient caries:

Abu Naba’a et al.20 conducted a split mouth randomised clinical trial. They included 90 participants with at least two primary pit or fissure lesions (without cavitation) in permanent posterior teeth accessible to diagnostic procedures. A total of 390 primary occlusal pit and fissure carious lesions were included in this study where 195 teeth received ozone gas and 195 did not. Included teeth were randomised to either ozone therapy (10 s of ozone gas at 2100 ppm concentration at the start of the study, falling to 630 ppm, only 30% of the full dose, by the end) or only cleaning (no ozone), though not always in equal numbers in every mouth. Of these samples, 132 lesions (66 from the ozone group and 66 from the control group) further received a fissure sealant.

For the unsealed teeth in both groups, caries progression and regression were measured by change in clinical severity scores, mean log(e) DIAGNOdent readings and mean log(e) ECM (electric caries meter) scores at baseline, 1, 3, 6, 9, and 12 months. For those sealed teeth, the quality of sealants was checked at each recall. Patient satisfaction was also assessed. They also requested participants to report their anxiety using a questionnaire and to compare ozone treatment to usual dental interventions including dental injection, drilling, filling, and scaling and polishing.

Their results showed that the mean changes in ECM readings were 0.337 and –0.065 for the treatment and control groups, respectively. At the first recall visit, the difference between groups was significantly better in the ozone group (p < 0.05). After the 3- and 6-month recall visits, the change in the clinical severity and the change in the DIAGNOdent scores were stabilised in the ozone group, respectively. At all recall visits these values were higher (worse) for the control group than for the ozone group, with a marginal (not statistical) significance of p = 0.081. In those sealed teeth, there was no significant difference in the short-term retention of sealants.

Using the anxiety questionnaire, they found that ozone treatment provoked the least state of anxiety comparing to traditional dentistry (p < 0.05). The anxiety was further reduced after the subjects received the ozone treatment (p < 0.05). Also, a high percentage of those happy with the ozone treatment (n = 89) were accompanied with satisfaction to receive the treatment again, paying for the costs, accepting time commitments, and even recommending it to a friend or relative.

With the same inclusion/exclusion criteria, the research group18 conducted a pilot study on eight participants with at least two primary pit or fissure caries (including cavitation) in permanent posterior teeth accessible to diagnostic procedures. They included a total of 38 primary occlusal pit and fissure carious lesions, which were individually randomised to two groups using a computer generated random tables:

Table 2 – Search strategy for literature review on the use of ozone in dentistry

<table>
<thead>
<tr>
<th>#</th>
<th>Search history</th>
<th>Hits</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Dentin or enamel or tooth or teeth or dentist$$</td>
<td>429320</td>
</tr>
<tr>
<td>2</td>
<td>Ozon$ or HealOzone</td>
<td>33974</td>
</tr>
<tr>
<td>3</td>
<td>1 and 2</td>
<td>199</td>
</tr>
<tr>
<td>4</td>
<td>Limit 3 to English</td>
<td>176</td>
</tr>
<tr>
<td>5</td>
<td>Remove duplicates from 4</td>
<td>154</td>
</tr>
<tr>
<td>6</td>
<td>Articles retrieved</td>
<td>42</td>
</tr>
</tbody>
</table>
(1) Nineteen lesions received ozone gas for 40 s at baseline, 1, 3, and 6 months.
(2) Another 19 lesions did not receive ozone.

For both groups, caries progression and regression were measured by the change in clinical severity scores, mean log(\(e\)) DIAGNODent readings, mean log(\(e\)) ECM scores and other clinical indices (surface destruction, hardness, visual index, colour of lesion, perceived treatment need, frostiness of enamel, and enamel undermining) at baseline, 1, 3 and 6 months. They found that significantly more lesions became arrested in the ozone group than the control group. They concluded that ozone treatment for 10 s significantly remineralised lesions and could be proposed as an alternative treatment for new pit or fissure lesions.

Recently, Huth et al.\(^\text{21}\) conducted a split mouth randomised controlled clinical study to evaluate the effect of 40 s ozone gas (HealOzone [KaVo Dental GmbH, Germany]) on the progression or reversal of non-cavitated initial occlusal fissure caries. They recruited 41 patients (mean age 7.7 ± 2.2 years) with 57 pairs of lesions on contra-lateral permanent molars and randomly assigned these teeth to two groups: the test tooth received the ozone treatment while the control tooth was left untreated. A calibrated blinded examiner monitored lesion progression or reversal using DIAGNODent for up to 3 months. Significantly more caries reversal or reduced caries progression was found in the ozone-treated lesions than in the untreated control lesions within the group of patients at high current caries risk. However, there was no statistical significance difference between the groups when the whole study population was examined.

### 3.1.2. Ozone in management of the open caries

One clinical study\(^\text{22}\) was found which evaluated the efficacy of ozone gas in the reversal of caries in open single-surface lesions: 82 open single-surface lesions in 28 apprehensive and non-treatable children (mean age 5.96 ± 2.36 years) were first excavated until a leathery consistency was recorded and then assigned to two groups (maximum two pairs per subject); the larger lesion was exposed to 20 s ozone gas (HealOzone) while the smaller lesion was left untreated as the control group.

Tactile hardness (hard, leathery and soft) and DIAGNODent readings were assessed and the changes for hardness and DIAGNODent values in the test lesion were compared with the values in the control lesion after 2, 4, 6, and 8 months.

Pair-wise comparison of the hardness between baseline and follow up showed the statistically significant improvement in the ozone-treated test lesions after 4, 6, and 8 months compared with baseline while the control lesions had no significant change in hardness at any recall interval. Comparing the differences between test and control teeth over time, the DIAGNODent values improved; however, the improvement was not statistically significant. Moreover, the use of ozone resulted in an average reduction of 13% of the DIAGNODent values immediately after the ozone treatment. Finally, with the use of questionnaire, it was found that the level of fear was reduced prior to the second session and following the last session by 82% and 93% of children, respectively, prior to the second session and following the last session.

### 3.1.3. Ozone in management of root caries

A total of three studies (one in vitro and two in vivo) were found to evaluate the preventive and therapeutic effect of ozone in the management of root caries:

In an in vitro study, Baysan et al.\(^\text{18}\) assessed the antimicrobial effect of aqueous ozone on primary root carious lesions (PRCLs). They harvested 40 soft PRCLs from freshly extracted teeth and removed half of a lesion with a sterile excavator. The remaining lesion was exposed to ozonated water for a period of either 10 or 20 s (0.069 or 0.138 mL of ozone, respectively). A significantly reduced microbiological count (\(p < 0.001\)) was observed in the ozone-treated groups compared with the control groups. They also used 40 sterile saliva-coated glass beads to demonstrate the specific antimicrobial efficacy of 10 s ozone gas (HealOzone) on Streptococcus mutans and Streptococcus sobrinus. The glass beads were randomly divided into two groups for each microorganism: ozone and control. They found a significant (\(p < 0.0001\)) reduction in ozone-treated samples for both microorganisms compared with the control samples.\(^\text{18}\)

In an in vivo setting, Baysan et al.\(^\text{23}\) conducted a split mouth randomised clinical trial. They included 79 participants, at least 18 years old, with either two or four primary root carious lesions (PRCLs). The included lesions were leathery (severity index two) and accessible to treatment. The lesions were randomised with equal numbers of intervention and control, in each mouth in a way that each participant had equal numbers of intervention and control lesions (two or four in total):

1. Cleaning, 10 s of ozone gas, and reductant solution (containing xylitol and fluoride). The intervention was repeated at 1 month (with no ozone), 3 months and 6 months (both with ozone for 10 s).
2. Reductant only (applied for 5 s), repeated after 1 month and 3 months.
3. Ozone + sealant. Ozone therapy was applied at 3 months. Sealants were reapplied if it was a partial or complete defect.
4. Sealant only.

For all groups caries progression and regression were measured by the change in clinical severity scores, mean log(\(e\)) DIAGNODent readings, and mean log(\(e\)) ECM scores. They also recorded further dental interventions, pain, patient satisfaction and adverse events.

Their results showed that in the 6-month follow up, the mean changes in ECM readings were significantly higher (i.e., better remineralization) in the ozone-treated group than in the control group (5.62 vs. 4.92, respectively). Also, DIAGNODent measurements in 6-month follow up were significantly lower (i.e., better remineralization) in the ozone-treated group than in the control group (10.9 vs. 46.4, respectively). In 12 months 47% of PRCLs became hard in the ozone group, while none became hard in the control group (\(p < 0.001\)). Fifty-two percent of lesions reversed from severity index of 2 to 1 in the ozone group compared with 11.6% in the control group (\(p < 0.001\)).

In another randomised clinical trial, Holmes\(^\text{12}\) evaluated the effect of ozone gas, combined with the daily use of a
remineralizing patient kit, on the clinical severity of non-cavitated leathery primary root carious lesions (PRCLs) in 89 subjects (age range 60–82). Each subject had two leathery PRCLs (a total of 178 lesions), which were randomly assigned for treatment with ozone gas (40 s at 2100 ppm ± 10%) or air. Lesions were clinically recorded at baseline, 3, 6, 12 and 18 months as soft, leathery or hard. He found that at 3 months, 69% of lesions in the ozone group had become hard and none had deteriorated. In the control group, after 3 months, 4% had become worse (p < 0.01). At the 6-month recall, 8% of lesions treated with ozone remained leathery and the rest had become hard. In contrast, in the control group, 11% of lesions had become worse and only one lesion had become hard (p < 0.01). At the 12-month recall, in ozone group, two lesions remained leathery and the rest had hardened. Over the same period, in the control group, 21 lesions had become worse, one remained hard, and the rest remained leathery (p < 0.01). At the 18-month recall, in the ozone group, all the lesions had arrested, while in the control group, 37% of the lesions had become worse, one had reversed, and the rest remained leathery (p < 0.01). In summary, this study reported that 100% of ozone-treated PRCLs had reversed by 18 months, while 37% of PRCLs in the control group had worsened from leathery to soft and 1% had reversed.

3.1.4. Methodological quality

In general there are some methodological concerns with these clinical studies:

Outcome measurement: It should be noted that the majority of the clinical studies that are reviewed depend on findings produced by the DIAGNOdent and ECM. These devices have not yet replaced visual, tactile and radiographic detection as a routine method of early caries diagnosis, although their validity and reliability have been extensively tested. Also, the clinical severity score (tactile hardness) used in four studies is subjective and could result in some level of measurement error.

Data analysis: In all these studies, the data analyses were conducted at the level of the lesion. The researchers assumed that there was no variability between subjects, i.e., all these teeth were analysed independently as if they were from different subjects. This approach may produce a significant result; however, the literature demonstrates its lack of validity.

Blinding: All these studies lack an appropriate method of blinding as no placebo ozone treatment was given and thus subjects and/or their parents were not blinded to the study. The exception is Holmes in which the allocation process was described as double-blinded. Blinding of outcome evaluators is unclear in two studies as the operator and the evaluator are one person at the same visit. However, this has been overcome in two studies where a calibrated and blind examiner (other than the operator) assessed the outcome. In the Holmes study, two dentists allocated subjects to intervention groups and assessed the severity of lesions, and the third dentist assessed the reproducibility of data. However, none were acknowledged in the paper or listed as co-authors. The details of the blinding is not provided in Dahnhardt et al.

3.1.5. Systematic reviews on ozone in clinical dentistry

In 2004, the Cochrane library hosted a systematic review to assess the effectiveness of ozone gas in arresting or reversing the progression of dental caries. The authors included three relevant randomised controlled trials (Abu Naba’a, Abu Naba’a Pilot, and Baysan studies as discussed above) with a combined total of 432 randomised lesions from 137 participants. They evaluated these studies at high risk of bias and concluded that reliable evidence is lacking that the application of ozone gas to the surface of decayed teeth stops or reverses the decay process and that there is not enough high quality evidence to support the use of ozone gas in a primary care setting.

Recently, Brazzelli et al. conducted a systematic review to assess the effectiveness of ozone gas (HealOzone) for the management of pit and fissure caries, and root caries. They reached a similar conclusion to that of the Cochrane library. They also constructed a Markov model to explore possible cost-effectiveness aspects of HealOzone in addition to current management of dental caries over a 5-year period. They found that treatment using current management plus HealOzone cost more than current management alone for non-cavitated pit and fissure caries (£40.49 vs. £24.78), but cost less for non-cavitated root caries (£14.63 vs. £21.45). They stated that the current evidence is insufficient to conclude that it is a cost-effective addition to the management and treatment of occlusal and root caries.

3.2. Secondary objective: a summary of the in vitro application of ozone in dentistry

3.2.1. Ozone in oral microbiology (Table 3)

Four studies were identified investigating the bactericidal effect of ozone in the oral cavity. Ozone might be useful to control oral infectious microorganisms in dental plaque. However, the evidence available is controversial: while some researchers found an incomplete efficacy of ozone (in aqueous or gaseous form) in eliminating the viable bacteria, the others found ozonated water effective for killing gram-positive and gram-negative oral microorganisms and oral Candida albicans. Also, a high level of biocompatibility of aqueous ozone on human oral epithelial cells, gingival fibroblast cells, and periodontal cells has been found. This is important for decontamination of avulsed teeth before replantation. Moreover, four studies were found that showed the in vitro antimicrobial efficacy of ozone as denture cleaners with ozone gas was found to be more effective than ozonated water. Therefore, gaseous ozone can be clinically useful for disinfection of dentures.
<table>
<thead>
<tr>
<th>Author, date</th>
<th>Objective</th>
<th>Methodology</th>
<th>Outcome measurements/results</th>
<th>Authors’ conclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Müller P et al., 2007⁴¹</td>
<td>To assess the antimicrobial potential of ozone gas and photodynamic therapy (PDT) in vitro</td>
<td>Mature six-species oral biofilms were treated as follows ($n = 9$ per group)</td>
<td>Treatment with chlorhexidine or hypochlorite served as a positive control, whereas untreated samples served as negative controls</td>
<td>Gasiform ozone and PDT had a minimal effect on the viability of microorganisms organised in a cariogenic biofilm</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1. Sixty-second application of gasiform vacuum-ozone or vacuum alone (on wet or air-dried biofilm samples)</td>
<td>Colony-forming units on blood agar: only the 5% hypochlorite solution was able to totally eliminate the microorganisms in the biofilm. The observed reduction of viable counts by vacuum-ozone application and PDT was less than 1 log₁₀ step</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2. PDT (i.e., methylene blue in combination with or without a diode soft laser, and a soft laser alone)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>3. Antimicrobial solutions: immersion of biofilms for 60 s in 0.2% and 2% chlorhexidine or in 0.5% and 5% hypochlorite solution</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Huth et al., 2006⁴³</td>
<td>To evaluate cytotoxicity of gaseous and aqueous ozone</td>
<td>Cytotoxicity of gaseous and aqueous ozone on human oral epithelial cells and gingival fibroblast cells compared with established antiseptics over a time of 1 min, and compared with the antibiotic, metronidazole, over 24 h</td>
<td>Cell counts, metabolic activity, Sp-1 binding, actin levels, and apoptosis were evaluated</td>
<td>Aqueous ozone revealed the highest level of biocompatibility of the tested antiseptics</td>
</tr>
<tr>
<td>Nagayoshi et al., 2004⁴⁷</td>
<td>To examine the effect of ozonated water on oral microorganisms and dental plaque</td>
<td>Dental plaque samples were treated with 4 mL of ozonated water for 10 s</td>
<td>No microorganisms were detected</td>
<td>Ozonated water was effective for killing gram-positive and gram-negative oral microorganisms and oral Candida albicans in pure culture as well as bacteria in plaque biofilm and therefore might be useful to control oral infectious microorganisms in dental plaque</td>
</tr>
<tr>
<td>Author, date</td>
<td>Objective</td>
<td>Methodology</td>
<td>Outcome measurements/results</td>
<td>Authors’ conclusion</td>
</tr>
<tr>
<td>------------------------------</td>
<td>---------------------------------------------------------------------------</td>
<td>------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
<td>-----------------------------------------------------------------------------------------------</td>
<td>-------------------------------------------------------------</td>
</tr>
<tr>
<td>Walker et al., 2003&lt;sup&gt;42&lt;/sup&gt;</td>
<td>To use an established biofilm laboratory model to simulate biofouling of dental unit water system</td>
<td>Stained bacterial cells were stained and fluorescence microscopic analysis was conducted to estimate the effect of ozonated water on Streptococcus mutans</td>
<td>S. mutans cells were killed instantaneously in ozonated water&lt;br&gt;Ozonated water significantly inhibited the accumulation of experimental dental plaque and remarkably decreased the number of viable S. mutans</td>
<td>While many disinfectants achieve a sufficient reduction in total viable counts they may not necessarily remove unwanted biofilm from the tubing surfaces</td>
</tr>
<tr>
<td>Ebensberger et al., 2002&lt;sup&gt;6&lt;/sup&gt;</td>
<td>To evaluate the effect of irrigation with ozonated water on the proliferation of cells in the periodontal ligament adhering to the root surfaces</td>
<td>They evaluated practicality, cost-effectiveness, and efficacy of different disinfectants (including ozone) compared to sterile water as a negative control</td>
<td>Ozone, as applied for 10 min, did not completely eliminate the viable bacteria (65% reduction in biofilm total viable counts) nor did it remove the biofilm (57% reduction in biofilm coverage)</td>
<td>Two-minute irrigation of the avulsed teeth with non-isotonic ozonated water might lead not only to a mechanical cleansing, but also decontaminate the root surface, with no negative effect on periodontal cells remaining on the tooth surface</td>
</tr>
<tr>
<td>Antimicrobial effects of ozone as denture cleaner&lt;br&gt;Estrela et al., 2006&lt;sup&gt;45&lt;/sup&gt;</td>
<td>To evaluate the antimicrobial potential of ozone applied to three different solutions in an ultrasonic cleaning system against Staphylococcus aureus</td>
<td>Twenty-three freshly extracted completely erupted third molars without antagonists in patients between 20 and 35 years of age</td>
<td>The labeling index (the number of positive cells compared to the total number of cells suggesting enhancement of metabolism) was higher among the teeth irrigated with ozone (7.8% vs. 6.6%); however, the difference was not statistically significant ($p = 0.24$)</td>
<td>The addition of ozone to an ultrasonic cleaning system containing different experimental solutions resulted in antibacterial activity against S. aureus</td>
</tr>
</tbody>
</table>

The addition of ozone to an ultrasonic cleaning system containing different experimental solutions resulted in antibacterial activity against S. aureus.
<table>
<thead>
<tr>
<th><strong>Arita et al., 2005</strong></th>
<th>To examine the effect of ozonated water on <em>C. albicans</em> on acrylic denture plate</th>
<th>Heat-cured acrylic resins were cultured with <em>C. albicans</em>, and treated with flowing ozonated water alone or in combination with ultrasonication</th>
<th>After exposure to flowing ozonated water (2 or 4 mg/L) for 1 min, there were no viable <em>C. albicans</em> cells</th>
<th>The application of ozonated water may be useful in reducing the number of <em>C. albicans</em> on denture plates</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Murakami et al., 2002</strong></td>
<td>To examine the bactericidal and virucidal effectiveness of a denture cleaner that uses ozone</td>
<td>Bactericidal and virucidal effectiveness of 10 ppm ozone (as an effective chemical of a commercial denture cleaner) against methicillin-resistant <em>S. aureus</em> and T1 phage</td>
<td>In the bactericidal activity test, with the ozone supply turned on, the number of bacteria was $3.1 \times 10^3$ CFU/mL before ozone treatment, 1.0 CFU/mL 10 min later, and 1.0 CFU/mL or less afterwards. Control comparison: the ozone supply was cut off to produce air bubbles. The number of bacteria was $3.4 \times 10^3$ CFU/mL at the beginning of the experiment, and fell to $3.0 \times 10^3$ CFU/mL 60 min later (no statistically significant difference). In the virucidal activity test, the number of phages was $1.2 \times 10^6$ PFU/mL at the beginning of the experiment, fell to about 1/10 of that number 10 min later, and was 6.1 PFU/mL 40 min later.</td>
<td>The use of ozone in this denture cleaner is effective against methicillin-resistant <em>S. aureus</em> and viruses</td>
</tr>
<tr>
<td><strong>Oizumi et al., 1998</strong></td>
<td>To compare the bactericidal effects of ozone in the aqueous and gaseous phases</td>
<td>Effect of gaseous ozone injection vs. ozonated water was evaluated on three standard strains of oral microorganisms: <em>S. mutans</em>, <em>S. aureus</em>, and <em>C. albicans</em></td>
<td>Consequent to applying gaseous ozone, the numbers of cells of all three strains decreased to 1/10 at 1 min</td>
<td>Direct exposure to gaseous ozone was a more effective microbicide compared with ozonated water, and that gaseous ozone can be clinically useful for disinfection of dentures</td>
</tr>
</tbody>
</table>

Sterile distilled water
Vinegar and sterile distilled water
Endozime AWplus

Consequent to applying gaseous ozone for 3 min, the numbers of cells of all three strains were below the detection limit
<table>
<thead>
<tr>
<th>Author, date</th>
<th>Objective</th>
<th>Methodology</th>
<th>Outcome measurements/results</th>
<th>Authors’ Conclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estrela et al., 2007</td>
<td>To determine the antimicrobial efficacy of ozonated water, gaseous ozone, 2.5% sodium hypochlorite and 2% chlorhexidine in human root canals infected by <em>Enterococcus faecalis</em></td>
<td>Thirty human maxillary anterior teeth were prepared and inoculated with <em>E. faecalis</em> for 60 days</td>
<td>No solution used as an irrigant over a 20-min contact time demonstrated an antimicrobial effect against <em>E. faecalis</em></td>
<td>The irrigation of infected human root canals with ozonated water, 2.5% NaOCl, 2% chlorhexidine and the application of gaseous ozone for 20 min was not sufficient to inactivate <em>E. faecalis</em></td>
</tr>
<tr>
<td>Hems et al., 2005</td>
<td>To evaluate the potential of ozone as an antibacterial agent using <em>E. faecalis</em> as the test species</td>
<td>The antibacterial efficacy of ozone was tested against both broth and biofilm cultures. Ozone was sparged for 30, 60, 120 and 240 s, through overnight broth cultures of <em>E. faecalis</em> and compared with those that were centrifuged, washed and resuspended in water</td>
<td>Significant reductions of bacteria in the unwashed (2 log 10 reductions) and washed (5 log 10 reductions) broth cultures following 240 s of ozone applications</td>
<td>NaOCl was found to be superior to ozonated water in killing <em>E. faecalis</em> in broth culture and in biofilms</td>
</tr>
<tr>
<td>Nagayoshi et al., 2004</td>
<td>Effect of ozonated water against <em>E. faecalis</em> and <em>S. mutans</em> infections in dentin of freshly extracted bovine incisors</td>
<td>After treating with ozonated water, the number of colony forming units (CFU) of bacteria in the infected dentin chips was significantly decreased</td>
<td>Ozonated water had nearly the same antimicrobial activity as 2.5% NaOCl during irrigation, especially when combined with sonication, and showed a low level of cytotoxicity against cultured cells</td>
<td>Ozonated water application might be useful for root canal irrigation</td>
</tr>
<tr>
<td>Author, date</td>
<td>Objective</td>
<td>Methodology</td>
<td>Outcome measurements/results</td>
<td>Authors’ conclusion</td>
</tr>
<tr>
<td>-------------</td>
<td>-----------</td>
<td>-------------</td>
<td>------------------------------</td>
<td>---------------------</td>
</tr>
<tr>
<td>Celiberti et al., 2006&lt;sup&gt;21&lt;/sup&gt;</td>
<td>To assess the effects of the highly reactive molecule of ozone on sound enamel physical properties and its effects on sealing ability</td>
<td>The effect of ozone gas (HealOzone, KaVo, 2100 ppm for 40 s) was evaluated prior to etching and sealing on sound enamel physical properties, including knoop surface microhardness, contact angle (free surface energy of enamel), acid resistance, and sealing ability</td>
<td>No statistically significant difference between the control and ozone-treated samples in all tests</td>
<td>Ozone can be applied as a prophylactic treatment prior to etching and the placement of sealant</td>
</tr>
<tr>
<td>Polydorou et al., 2006&lt;sup&gt;49&lt;/sup&gt;</td>
<td>To evaluate the antibacterial effect of two different application times of ozone gas on an in vitro infected cavity model, comparing to the already proven activity of two dentin bonding agents</td>
<td>Thirty-five extracted human molars were divided into five groups (control, Clearfil SE Bond, Clearfil Protect Bond, 40 s of ozone, 80 s of ozone) and 28 cavities were prepared in each group</td>
<td>All four treatments significantly reduced the number of S. mutans compared with the control group and that the antimicrobial effect of both bonding systems and 80 s of ozone was significantly higher than the 40 s ozone treatment</td>
<td>Longer exposure to ozone has a strong bactericidal effect on microorganisms within the dentinal tubules of deep cavities, which could result in increasing the clinical success of restoration</td>
</tr>
<tr>
<td>Schmidlin et al., 2005&lt;sup&gt;50&lt;/sup&gt;</td>
<td>To evaluate the influence of direct high-dose gaseous ozone application on dentin and enamel shear bond strength</td>
<td>Ten bovine enamel and dentin samples per group were pretreated as follows</td>
<td>After storage in water at 37°C for 24 h, shear bond strength was measured using a Zwick universal testing machine</td>
<td>Despite a possible retention of surface and subsurface oxide-related substances during high-dose ozone application, shear bond strength was not impaired. Thus, adhesive restoration placement should be possible immediately after ozone application for cavity disinfection</td>
</tr>
</tbody>
</table>

1. Ozone application (HealOzone, KaVo) for 60 s alone
2. With subsequent application of a fluoride- and xylitol-containing antioxidant (liquid reductant)
3. Light-activated bleaching with 35% hydrogen peroxide for 5 min serving as negative control

Bleaching resulted in significantly decreased bond strength (p < 0.05) on enamel specimens. No decrease in shear bond strength was detected for ozone-pretreated specimens compared to untreated controls
3.2.2. Ozone in endodontics (Table 4)
Four in vitro studies\(^5,41,47,48\) were identified investigating the bactericidal effect of ozone as compared to 2.5% sodium hypochlorite, the standard irrigation solution in endodontics. The results on this outcome are controversial: while Nagayoshi et al., 2004\(^5\) found nearly the same antimicrobial activity (against Enterococcus faecalis and S. mutans) and a lower level of cytotoxicity of ozonated water as compared to 2.5% NaOCl; in a study by Hems et al., \(^48\) NaOCl was found to be superior to ozonated water in killing E. faecalis in broth culture and in biofilms while gaseous ozone had no effect on the E. faecalis biofilm. Muller et al., 2007\(^41\) has also found 5% NaOCl superior to gaseous ozone in eliminating microorganisms organised in a cariogenic biofilm. Moreover, a recent study has found that the irrigation of infected human root canals with ozonated water, 2.5% NaOCl, 2% chlorhexidine and the application of gaseous ozone for 20 min was not sufficient to inactivate E. faecalis.\(^47\)

3.2.3. Ozone in prosthodontics (Table 5)
There were three retrieved studies\(^11,49–51\) that assessed the efficacy of ozone in restorative dentistry and its effect on dental materials. The highlights of these studies are as follows:

1. Ozone gas can be applied as a prophylactic treatment prior to etching and the placement of sealant with no negative impact on sound enamel physical properties, including Knoop surface microhardness, or contact angle.\(^11\)

2. The longer exposure to ozone gas has a strong bactericidal effect on microorganisms within the dentinal tubules of deep cavities, which could result in increasing the clinical success of restorations,\(^49\) with no negative impact on dentin and enamel shear bond strength to adhesive restoration.\(^50\)

3. Ozone can be applied for cleaning the surface of removable partial denture alloys with little impact on the quality of alloy in terms of reflectance, surface roughness, and weight.\(^51\)

There is also some evidence on the effectiveness of aqueous ozone application in adjunct to amino–alcohol for decontamination of the implant surfaces.\(^52\)

3.2.4. Ozone in oral and maxillofacial surgery
One study was found to evaluate the effect of ozone gas in oral and maxillofacial surgery, where ozone therapy was found to be beneficial for the treatment of the refractory osteomyelitis in the head and neck in addition to treatment with antibiotics, surgery and hyperbaric oxygen.\(^53\) Other studies on this area are mostly non-English publication and were reviewed elsewhere.\(^54\)

4. Conclusion
A thorough literature review on the application of ozone in dentistry has identified these concluding notes:

1. The clinical studies identified in the literature review focused on the application of ozone gas in the management of primary occlusal and root carious lesions. Despite the good evidence in in vitro settings, the clinical application of ozone has not achieved strong level of efficacy and cost-effectiveness.
(2) There is good evidence of in vitro biocompatibility of aqueous ozone with human oral epithelial cells, gingival fibroblast cells, and periodontal cells.\textsuperscript{6,43}

(3) There is conflicting evidence of antimicrobial efficacy of ozone in in vitro settings.\textsuperscript{6,7,41,42} However, there is some evidence that ozone (in both gaseous or aqueous phase) is a potentially effective disinfectant agent for removing the biofilms and the related microorganisms such as Legionella pneumophila, Mycobacterium spp., Pseudomonas aeruginosa, and Candida spp. from dental unit water system and an effective bactericidal agent for removing \textit{S. mutans}, methicillin-resistant \textit{Staphylococcus aureus}, \textit{Candida albicans}, and \textit{E. faecalis} from dentures.\textsuperscript{9,44–46}

(4) There is conflicting evidence in the in vitro application of ozone in endodontics.\textsuperscript{3,42,47,48}

(5) There is good evidence of in vitro application of ozone as a useful prophylactic antimicrobial treatment prior to etching and the placement of dental sealants and restorations with no negative interaction with the physical property of enamel and adhesive restorations.\textsuperscript{11,49,50}

(6) There is good evidence of the in vitro application of ozone as a denture cleaner with little impact on the quality of denture alloy in terms of reflectance, surface roughness, and weight.\textsuperscript{9,44–46,51}

(7) There is insufficient evidence in the application of ozone in oral and maxillofacial surgery, oral diseases, and implant therapy.

In conclusion, this review has demonstrated that while laboratory studies suggest a promising potential of ozone in dentistry, the clinical evidence for application of ozone in dentistry is not extensive. There is still a need for the highest level of evidence, i.e., well designed, double-blind randomised clinical trials with adequate sample size, limited or no loss to follow up, and carefully standardised methods of measurement and analysis in order to justify the routine use of ozone as a treatment modality in dentistry.

Acknowledgements

The authors would like to thank Dr. Edward D. Fillery, Department of Oral Microbiology and Dr. Herenia P. Lawrence, Department of Community Dentistry, Faculty of Dentistry, University of Toronto for proof reading the manuscript.

REFERENCES

15. Bocci V. Ozone as Janus: this controversial gas can be either toxic or medically useful. \textit{Mediators of Inflammation} 2004;13:3.


42. Walker JT, Bradshaw DJ, Fulford MR, Marsh PD. Microbiological evaluation of a range of disinfectant products to control mixed-species biofilm contamination in a laboratory model of a dental unit water system. Applied & Environmental Microbiology 2003;69:3327.


