

# Ask the Experts

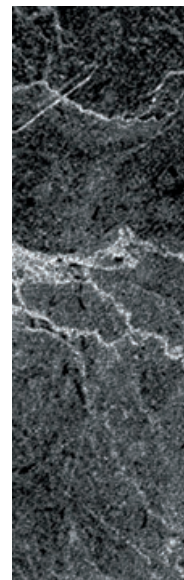
## EVIDENCE-BASED EFFICACY OF OZONE FOR ROOT CANAL IRRIGATION

### Guest Expert

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### Associate Editor

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**QUESTION:** As a follow-up to the recently published information on ozone as a means of caries treatment, can you provide some information on the use of ozone in root canal therapy?

**ANSWER:** Ozone has been proposed as a dental antiseptic agent based on reports of its antimicrobial effects in both gaseous and aqueous forms. Ozone is effective when it is prescribed in sufficient concentration, used for an adequate time, and delivered correctly into root canals after the traditional cleaning, shaping, and irrigation have been completed. Ozone will not be effective if too little dose of ozone is delivered or it is not delivered appropriately.

Ozone should be used after the conventional cleaning, shaping, and irrigation of root canals, and the ozonated liquid in the canal system should be agitated with ultrasound.

#### PROVEN ANTIMICROBIAL EFFICACY OF OZONE

Ozone is one of the most powerful antimicrobial agents available for use in medicine or dentistry.<sup>1</sup> As failure of root canal therapy is mainly caused by microorganisms, it is not surprising that there are enormous advantages to killing these pathogens. Numerous peer-reviewed published research papers have proven the antimicrobial effectiveness of ozone as a gas and as ozonated water.<sup>2-20</sup>

In model dental unit water lines, ozone achieved a 57% reduction in biofilm and a 65% reduction in viable bacteria in spite of being used in a very low dose and with a short time of application.<sup>21</sup> Ozone rapidly kills otherwise hard to kill microorganisms.

#### RECOMMENDED USE OF OZONE IN ROOT CANAL THERAPY

Ozone works best when there is less organic debris remaining. Therefore, the recommendation is to use either ozonated water or ozone gas at the end of the cleaning and shaping process. I personally still use my conventional irrigants during this earlier phase and I finally irrigate with ozonated water (TherOzone, Santa Monica,

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CA, USA) using ultrasonics. I also bubble ozone gas (HealOzone, KaVo, Biberach, Germany) into this ozonated water and use ozonated oil (Lime Technologies Ltd., Capetown, South Africa) as a medicament.

#### COMPARISON OF THE USE OF OZONE AND SODIUM HYPOCHLORITE IN ROOT CANAL THERAPY

Oxygen has a dramatically toxic effect to microaerophilic and anaerobic bacteria. Virtej and colleagues<sup>22</sup> compared the antimicrobial performance of four systems used as root canal irrigants. Seventy instrumented and initially sterile roots with open access cavities and containing a paper point were carried by one volunteer in the oral cavity for 1 week. After removal, the samples were taken for microbiologic analysis. The root canals were then disinfected with the Endox Endodontic System (Lysis S.r.l., Nova Milanese [MI], Italy), MTAD (Dentsply Tulsa Dental, Tulsa, OK, USA), 3% sodium hypochlorite (NaOCl), or HealOzone, and thereafter, the samples were repeated for microbiologic analysis. The roots were then sealed and incubated for a further week, after which bacterial growth was again determined. After disinfection, there was a significant decrease in the absolute bacterial count between each disinfection method and the positive

control group. There was no statistically significant difference between the 3% NaOCl, MTAD, and HealOzone groups. The Endox device showed the least antibacterial effect with significant differences to MTAD and HealOzone. Bacterial regrowth after 1 week of incubation was detected in all specimens of the control group, whereas the test groups showed several bacteria-free specimens. The authors concluded that ozone has great potential in endodontic antimicrobial use and that MTAD and HealOzone seem to be as effective as 3% NaOCl in reducing mixed bacterial infection in the root canal system.<sup>22</sup>

I would speculate that the antimicrobial effect of the ozone would have been even greater if it had been used as I recommended above. I personally feel that conventional irrigation (including NaOCl) should be used during cleaning and shaping, and ozonated water (ideally with ozone gas) should be used as the final irrigant with ultrasonication.

Cardoso and colleagues<sup>2</sup> concluded that the ozonated water, used as an irrigant agent, significantly reduced the number of *Candida albicans* and *Enterococcus faecalis* in root canals in human teeth.

A review<sup>23</sup> identified four studies<sup>6,24–26</sup> investigating the

bactericidal effect of ozone as compared with 2.5 to 5% sodium hypochlorite as irrigation solutions in endodontics.

Nagayoshi and colleagues<sup>6</sup> found nearly the same antimicrobial activity (against *E. faecalis* and *Streptococcus mutans*) and a lower level of cytotoxicity of ozonated water as compared with 2.5% NaOCl. They stated, “Ozone is known to act as a strong antimicrobial agent against bacteria, fungi, and viruses. In the present study, we examined the effect of ozonated water against *Enterococcus faecalis* and *Streptococcus mutans* infections *in vitro* in bovine dentin. After irrigation with ozonated water, the viability of *E. faecalis* and *S. mutans* invading dentinal tubules significantly decreased. Notably, when the specimen was irrigated with sonication, ozonated water had nearly the same antimicrobial activity as 2.5% sodium hypochlorite (NaOCl). We also compared the cytotoxicity against L-929 mouse fibroblasts between ozonated water and NaOCl. The metabolic activity of fibroblasts was high when the cells were treated with ozonated water, whereas that of fibroblasts significantly decreased when the cells were treated with 2.5% NaOCl. These results suggest that ozonated water application may be useful for endodontic therapy.”<sup>6</sup>

Muller and colleagues<sup>24</sup> found 5% NaOCl superior to gaseous ozone in eliminating microorganisms organized in a cariogenic biofilm. This study reported less than one log reduction of bacteria after using ozone gas above biofilms in culture media, which was only a similar reduction to that achieved by using 0.2% chlorhexidine or photoactivated disinfection.<sup>24</sup> However, it should be noted that ozone is a potent oxidant and will undergo a redox reaction with reductants in a culture media. In addition, the authors did not bubble the ozone into the biofilm. Ozone should be delivered under pressure into a root canal irrigant or lesion by pressing the delivery tube onto the surface so that ozone can penetrate the root canal irrigant or lesion. In vivo root canal contents and caries, unlike artificial biofilms, contain many molecules such as iron, which can increase the antimicrobial effectiveness of ozone in teeth and can help produce the powerful hydroxyl radicals in vivo to further increase the antimicrobial effectiveness of ozone.

Moreover, another study<sup>25</sup> has found that the irrigation of infected human root canals with ozonated water, 2.5% NaOCl, 2% chlorhexidine, or the application of gaseous ozone was not sufficient to inactivate *E. faecalis*. The methodology used was obviously spartan,

as no tested agent had any antimicrobial effect. It is highly probable that the ozone (oxidant) reacted preferentially with the reductants in the brain–heart infusion used for the inoculation in a simple redox reaction rather than with the bacterial strain.

Hems and colleagues<sup>26</sup> concluded that “ozone had an antibacterial effect on planktonic *E. faecalis* cells and those suspended in fluid, but little effect when embedded in biofilms. Its antibacterial efficacy was not comparable with that of NaOCl under the test conditions used.” Unfortunately, these authors used an extremely low dose of ozone in their experiments. The concentration of ozone mentioned in the paper was only 0.68 ppm. This concentration was immediately after production and would have reduced further by the time it was used. This was clearly a biased comparison as the dose of NaOCl used was enormous in comparison to the ozone. Surprisingly, immediately following ozone sparging, 1 mL of this broth had ozone inactivation by a transfer into 9 mL of neutralizing broth. This neutralization does not appear to have been similarly used with the NaOCl, again biasing the experiment. Given the methodology used in this paper, and the low dose and time of application of ozone used, it is surprising that ozone was as effective as it was reported.

#### USE OF OZONATED OILS AS MEDICAMENTS

An investigation evaluated histologically and histobacteriologically the response of periradicular tissues to the endodontic treatment of infected root canals performed in a single visit or in two visits using either ozonated oil or calcium hydroxide in camphorated paramonochlorophenol (CMCP) as an intracanal medication.<sup>27</sup> After 6 months, the animals were sacrificed and the specimens were processed for histologic and histobacteriologic analyses. The root canals treated in a single visit showed a success rate of 46%. When a calcium hydroxide/CMCP-based interappointment intracanal medication was used, 74% of the cases were categorized as successful. In cases where ozonated oil was used as the intracanal medication, the success rate was 77%.

Siqueira and colleagues<sup>28</sup> evaluated the antibacterial activity of the ozonated oil and calcium hydroxide pastes against bacterial species commonly associated with the etiology of periradicular diseases. Of the tested medicaments, ozonated oil was the most effective against the evaluated bacterial species.

#### BIOCOMPATIBILITY OF OZONE IN ROOT CANAL THERAPY

A high level of biocompatibility of aqueous ozone on human oral

epithelial (BHY) cells, gingival fibroblast (HGF-1) cells, and periodontal cells has been published.<sup>6,29-32</sup>

Huth and colleagues<sup>29</sup> investigated whether gaseous ozone and aqueous ozone exerted any cytotoxic effects on BHY cells and HGF-1 cells compared with established antiseptics (2 and 0.2% chlorhexidine digluconate [CHX]; 5.25 and 2.25% sodium hypochlorite [NaOCl]; 3% hydrogen peroxide [H<sub>2</sub>O<sub>2</sub>]) over 1 minute and compared with the antibiotic metronidazole over 24 hours. 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. CHX (2%, 0.2%) was highly toxic to BHY cells, and slightly toxic (2%) and nontoxic (0.2%) to HGF-1 cells. NaOCl and H<sub>2</sub>O<sub>2</sub> resulted in markedly reduced cell viability (BHY, HGF-1), whereas metronidazole displayed mild toxicity only to BHY cells. Taken together, aqueous ozone had the highest level of biocompatibility of the tested antiseptics. Nonetheless, ozone gas performed well compared with the established endodontic irrigants, which showed equal or even higher cytotoxic potentials than ozone gas. In addition, ozone gas applied into the

moist root canal, as currently performed with the HealOzone device, dissolves in canal fluids, thereby resulting in aqueous ozone, which then comes into contact with tissues.

Other reports also reported a high biocompatibility of aqueous ozone. Irrigation of the root surface of avulsed teeth did not reveal a negative effect on periodontal ligament cell proliferation.<sup>30</sup> A clinical report regarding the healing-accelerating effect of ozonated water did not document detrimental effects on cells.<sup>31</sup>

#### EFFECT OF AQUEOUS OZONE ON THE NF- $\kappa$ B SYSTEM

The transcription factor NF- $\kappa$ B plays a crucial role in inflammatory/immune processes and apoptosis. NF- $\kappa$ B is also thought to be of primary importance in the regulation of periodontal/periapical inflammatory reactions and the pathogenesis of periodontal diseases and apical periodontitis. Huth and colleagues<sup>32</sup> reported that aqueous ozone exerts inhibitory effects on the NF- $\kappa$ B system, suggesting that it has anti-inflammatory and immune-modulatory capacities.

#### OZONE IS A POTENT OXIDIZER

Ozone has been proven to be one of the most powerful oxidants we can use in dentistry.<sup>33</sup>

#### OZONE SYSTEMS AVAILABLE FOR USE IN ROOT CANAL THERAPY

KaVo produces the HealOzone, which delivers 2,100 ppm ozone at a flow rate of 615 cc per minute and has been proven to be safe.<sup>34,35</sup> TherOzone produces an excellent unit to produce ozonated water for root canal irrigation and numerous other applications. In addition, other systems are available (such as that supplied by Lime Technologies) that blow ozone into root canals, but manufacturer's directions must be followed in order to prevent any potential lung inhalation. Lime Technologies also sells ozonated oils for use as root canal medicaments.

#### USE OF OZONE TO MANAGE ANY CARRIES REMAINING IN THE ACCESS CAVITY

Ozone has been proven to help reduce cariogenic microorganisms and this could be beneficial to reduce potential contamination of the canal systems during instrumentation.<sup>20,36-49</sup>

#### ENHANCED HEALING ASSOCIATED WITH OZONE USE

Ozone also can play a key part in the healing process.<sup>6,29-32,50-59</sup>

#### CONCLUSION

Of course, more research on the use of ozone in root canal therapy will add to our knowledge in endodontics.

Thousands of dentists worldwide use ozone in root canal therapy and it is claimed that millions of teeth have received root canal therapy with ozone having been used as the final irrigant. No adverse event has been recorded after use of the HealOzone or ozonated water in root canal therapy.

Ozone is an effective, easy, cheap, and fast treatment to help disinfect root canals. Ozone is much stronger than chlorine and acts 3,000 times faster without producing harmful decomposition products.<sup>60</sup>

As ozone is the most powerful antimicrobial and oxidant we can use in endodontics, and as aqueous ozone revealed the highest level of biocompatibility compared with commonly used antiseptics, then it is fairly obvious that ozone should be used to help combat the microorganisms associated with infected root canals.

Ozone has a place in the 21st century oral health care,<sup>61</sup> and we should use its proven powerful antimicrobial efficacy and potent oxidant ability to reduce microorganisms during root canal therapy.

#### DISCLOSURE

Professor Edward Lynch is a consultant and principal investigator

for research grants from CurOzone USA (Aurora, Ontario, Canada) administered by Queens University, Belfast, Northern Ireland, UK.

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