Evaluation of unique property of ozone in comparison with 3% sodium hypochlorite in eradication of enterococcus faecalis

Abstract
Background: The success of endodontic treatment depends on complete sterilization of the root canals. Aim: To compare the efficacy of ozone and 3% sodium hypochlorite in eradication of Enterococcus Faecalis. Materials and Methods: The sample consists of 30 retreatment cases of single rooted anterior teeth, which were divided into three groups of 10 each. Group 1 was subjected to sparing with gaseous ozone. Group 2 was subjected to irrigation with aqueous ozone; group 3 was subjected to irrigation with 3% sodium hypochlorite. Samples were collected and were cultured on Mcconkey agar media. Enterococcus faecalis was isolated and counted on colony forming unit. Results: Reduction in bacterial colony count was seen in patients treated with aqueous ozone and 3% sodium hypochlorite, while minimal reduction was observed on samples of gaseous ozone. Conclusion: The study concluded that gaseous ozone does reduce number of E. faecalis in planktonic form and had a minimal effect on E. faecalis biofilm. The aqueous ozone and 3% sodium hypochlorite was more effective in reducing E. faecalis Strain in planktonic phenotype.
Key Words: Aqueous ozone; Enterococcus faecalis; Gaseous ozone; Sodium hypochlorite

Introduction
The success of root canal treatment depends on complete sterilization of root canals. This objective becomes more difficult to achieve as root canal system is polymicrobial in nature. Among the various microorganisms which are responsible for the failure of the root canal treatment, Enterococcus Faecalis holds an important position and is also used as a biological marker. (1, 2) Its occurrence in root filled teeth with periradicular lesions ranges from 24% to 77% and the most important criteria during chemo mechanical preparation is to include an irrigating system which can eradicate E. Faecalis. (3) The aim of this in vivo study was to investigate the antibacterial effectiveness of ozone using a single strain of Enterococcus faecalis, in order to establish its potential as a root canal disinfectant.

Materials & Methods
The study was conducted in Datta Meghe Institute of Medical Sciences, India. Thirty healthy individuals under the age group of 25-30 years, who required retreatment of previously performed root canal therapy, were included in the study. After rubber dam application in complete aseptic conditions Gutta-percha was removed by hand instrumentation and apical access was obtained. The patients were divided into three groups based on the solution used for irrigating the canal.

In group 1 an ozone-generating device was used to deliver gaseous ozone at the rate of 5.8 cm³ atmospheric pressure and 21°C for 60 sec through a nozzle connected to the device into the orifice of root canal. In group 2, freshly prepared ozonated water was used as an irritants. In group 3, 3% sodium hypochlorite (NaOCl) was used. Root canal isolate was taken with the help of absorbent points, carried to the laboratory in nutrient broth, samples were vortexed and tenfold dilution was done and cultured. Counting was done on colony forming unit, for all groups, same procedures were repeated.

Laboratory Procedure: The growth of facultative bacteria Enterococcus faecalis, samples were inoculated on culture plate of Mcconkey medium and incubated at 35 ± 2°C for 18-24 hours and up to 48 hours. Pinkish colonies were observed on the media, Bile Test was done to confirm facultative Enterococcus and confirmation of E. Faecalis done by Catalase Test. Gram staining of colony was done for microscopic examination and E. Faecalis was confirmed in culture. Cultured Mcconkey agar plates were mounted on electronic colony forming unit measurement chamber and colonies were counted. The effect of gaseous, aqueous ozone & sodium hypochlorite on bio-films was investigated.

Results
The effect of aqueous, gaseous ozone and sodium hypochlorite on Enterococcus faecalis in biofilm culture was evaluated and
Discussion

A number of different approaches to eliminating infection from root canal systems have been proposed including: the non-instrumentation technique, laser technology, irrigation with electrochemically activated Water and application of ozone. Ozone (O₃) is a powerful oxidizing agent and has been used in the water industry for many years to kill bacteria. In the dental field ozone has been advocated for treatment of gum infections, during surgery, for failed implant cases, root canals and root canal treatment. It is also postulated that ozone will penetrate through apical foramina and enter into surrounding and supportive bone tissue. The effect of ozone on these tissue will be to encourage healing & regeneration.

Sundqvist et al recovered numerous species of anaerobic bacteria from failed root canal systems. Results of the study showed that 38% of failed root canal treated teeth were contaminated by the bacteria Enterococcus faecalis. E. Faecalis in dentinal tubules has been shown to resist intracanal dressings of calcium hydroxide for over 10 days. As a result, antibacterial irrigants have to be relied upon to penetrate to the non-instrumented surfaces. Ozone is one of nature's most powerful oxidants, and directly oxidizes the substrate through decomposition via OH ions.

In accordance to Nagayoshi et al, Gulabivala et al & Estrela et al ozonated water had nearly the same antimicrobial activity as 2.5% NaOCl during irrigation. Our study shows similar results. Escherichia coli cells however, have been found to be inactivated primarily by molecular ozone. The absorbance of ozone in the water increased almost linearly with time, from 5 to approximately 60s. The stability of the ozone in the water was low and the ozone dissipated very quickly in ozone demand-free water at room temperature over 5 min, in agreement with Schechter. Therefore, Ozonated water was also used as the medium for testing and as a comparison. The antimicrobial nature of ozone was tested on E. faecalis cultured from planktonic forms. The bactericidal effects of 3% NaOCl on planktonic bacteria were used as the positive control and compared with the effects of ozone. Although 0.5–5% NaOCl are commonly used for chemo-mechanical cleaning of root canals, it was impossible to make direct comparisons between ozone and NaOCl because the concentration of ozone changes constantly during continuous spraying. In contrast, the higher concentration of 3% NaOCl specially for endodontic purpose was used as a positive control for the studies on E. faecalis biofilms based on a previous study, although no previous data were found on biofilm inhibitory concentration. The use of a bacterial biofilm grown on a simple membrane eliminates the variable of root canal anatomy and reduces the variations in quantity of growth contact of the biofilm with the antimicrobial agent. In this study Gaseous ozone was found to be less effective against E. faecalis as compare to aqueous ozone.

Conclusion

Within the limitations of this study, the following conclusions may be drawn about ozone’s ability to kill E. faecalis. a) Gaseous ozone does reduce number of E. Faecalis in planktonic form but does not illuminate it, b) Gaseous ozone had minimal effect on E. faecalis biofilm, and c) Aqueous ozone & 3% sodium hypochlorite was more effective in reducing E. faecalis Strain in planktonic phenotype.

Acknowledgements: This study was supported, in part, by Central Ethics Committee on Human Research, Datta Meghe Institute of Medical Sciences. The authors wish to thank staff member of department of General Microbiology and Oral pathology and Microbiology of Dnims University, Wardha, India. For their assistance with providing all laboratory facility and technical help.

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>Std. Error</th>
<th>95% Confidence Interval for Mean Lower Bound</th>
<th>Upper Bound</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gaseous Ozone</td>
<td>10</td>
<td>6.41 x 10^5</td>
<td>9.73 x 10^5</td>
<td>3.07 x 10^5</td>
<td>5.71 x 10^5</td>
<td>7.10 x 10^5</td>
<td>4.90 x 10^5</td>
<td>7.90 x 10^5</td>
</tr>
<tr>
<td>Aqueous Ozone</td>
<td>10</td>
<td>6.02 x 10^5</td>
<td>10.6 x 10^5</td>
<td>3.35 x 10^5</td>
<td>5.26 x 10^5</td>
<td>6.77 x 10^5</td>
<td>4.40 x 10^5</td>
<td>7.80 x 10^5</td>
</tr>
<tr>
<td>Control with NaOCl</td>
<td>10</td>
<td>5.02 x 10^5</td>
<td>10.6 x 10^5</td>
<td>3.35 x 10^5</td>
<td>4.26 x 10^5</td>
<td>5.77 x 10^5</td>
<td>3.40 x 10^5</td>
<td>6.80 x 10^5</td>
</tr>
</tbody>
</table>

Table 1: Comparison of E. Faecalis colonies in Gaseous and Aqueous ozone with control (with NaOCl) group.
Authors Affiliations: 1. Dr. Ajay Saxena, Associate Professor, 2. Dr. Rohan Bhede, Postgraduate Student, 3. Dr. Manoj Chandak, Professor and HOD, 4. Dr. Narendra Manwar, Professor, 5. Dr. Pradnya Nikhade, Associate Professor, Department of Conservative Dentistry and Endodontics, Sharad Pawar Dental College and Hospital, Wardha, Maharashtra, India.

References

Address for Correspondence
Dr. Ajay Saxena, MDS, Associate Professor, Department of Conservative Dentistry, Sharad Pawar Dental College and Hospital, Wardha, Maharashtra, India.

Source of Support: Nil, Conflict of Interest: None Declared