

Effectiveness of Ozone against E.Faecalis in a Root Canal Suspension - An In-Vitro Study.

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Abstract:

Aim: To assess the antimicrobial efficacy of aqueous and gaseous ozone as an alternative antiseptic against endodontic pathogen *Enterococcus faecalis* in suspension.

Methodology: *Enterococcus faecalis*, were grown in Mc conkey media in planctonic phenotype. Cultures were exposed to ozone gas, aqueous ozone & sodium hypochlorite (NaOCl; 0.5%), (control) for 1 min and the colony forming units were determined before and after exposure. Statistical analysis used: Data were analyzed by one way ANOVA with Tamhane post hoc tests to compare independent samples ($\alpha=0.05$).

Results: Concentrations of gaseous ozone for 1 min/53ug almost and aqueous ozone 1min/20ug completely eliminated the suspended microorganisms as did 0.5% NaOCl. **Conclusion:** High-concentrated gaseous and aqueous ozone was dose-, strain- and time-dependently effective against the tested microorganisms in suspension.

Keywords: antimicrobials, biofilm, endodontics, microbiology, ozone, root canal.

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I. Introduction

Successful endodontic treatment comprises of eradicating or substantially reducing the microbial load in the root canal system¹. The treatment of apical periodontitis involves elimination of root canal infection by a combination of mechanical and chemical means stated that Mechanical instrumentation alone may only reduce the numbers of bacteria from the root canal system by 50 %. According to Ingle goals of irrigation include lavage of debris, tissue dissolution, antibacterial action and lubrication. But also exhibit relatively no cytotoxicity toward periapical & oral mucosa. Sodium hypochlorite (NaOCl) was first advocated as an important adjunct to proper biomechanical instrumentation, debridement & disinfection² and is the current irrigant of choice due to its antibacterial³ and tissue-dissolving effects⁴. However it has only a limited effect on the dissolution of smear layer and dentine. It is also cytotoxic to the periapical tissues.

Minimally Invasive Dentistry is now the new Standard of Care in all disciplines of dentistry, most importantly in preventive and operative dentistry. A number of different approaches to eliminating infection from root canal systems have been proposed including; the non-instrumentation technique⁵, laser technology, irrigation with electrochemically activated water⁶ and application of ozone.

Ozone is currently being discussed as a possible alternative antiseptic agent in endodontics because of its reported high antimicrobial power without the development of drug resistance Ozone is a form of oxygen, (O₃) referred to as activated oxygen, tri-atomic allotropic form of oxygen with a sharp, clean smell, produced by an electrical discharge in atmosphere. It is a powerful oxidizing agent (Broadwater et al. 1973) and has been used in the water industry for many years due to its high germicidal effectiveness - wide range of pathogenic organisms including bacteria, protozoa & viruses. It has also been used in medicine for the enhancement of circulation, stimulation of oxygen metabolism and activation of the immune system.

Amongst 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. 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*⁷. It is a persistent organism that is known to play a major role in the aetiology of periradicular lesions after root canal treatment⁷. Its occurrence in root filled teeth with periradicular lesions ranges from 24% to 77%⁸. Hence in this current study, *Enterococcus faecalis* was chosen as the test species for this experiment.

II. Materials and Methods

Test microorganism

Cases of Re-treatment were chosen as test subjects, rubber dam was applied, and swabs from the canal were taken with absorbent points, with the subjects consent and were transported in culture bulbs. Vortexed (Remi.India) in 1ml of peptone water and sonication (Ioba life.India) was done. Ten fold dilution was done, centrifuged and resuspended in PBS to a turbidity of McFarland 1 (3×10^8 colony forming units) diluted 1: 3 and culture was obtained on McConkey medium. There of a mix culture (fig 1) was acquired from which small pin-point colonies were stained and confirmed gram⁺ ve. Biochemical tests were done in which bile tests (fig 2) proved to be catalase positive, which confirmed *Enterococcus faecalis* and then the pure form (fig 3) was recultivated on McConkey agar plates.

Test agents

Ozone in both its forms, gaseous form (protocol 1) (fig 4) with a concentration of 53 gm^{-3} for 1min and aqueous form (protocol 2) (fig 5) 1ml for 1min were compared with freshly prepared solutions of NaOCl (0.5%) (protocol 3) or control group. the colony forming units of all the protocols were determined before & after the exposure (fig 6 & 7). Ozone was produced by a custom made bench top generator, *ozoneDTA .nagayoshi, Japan*. and its aqueous form was acquired from alkachem, India. The antibacterial efficacy of ozone was tested against broth cultures. Preparation of all glassware used throughout this study was carried out by thoroughly rinsing with ozonated water.

Enterococcus faecalis broth cultures were sparged with gaseous ozone at concentration of 53 g m^{-3} for 1 min (protocol 1), aqueous ozone of 1ml for 1 min (protocol 2) & 0.5% NaOCl for 1min (protocol 3) streaked on another petridish for checking their viability and incubated for 24 hrs at 37°C and colony forming units determined.

III. Results Statistical Analysis

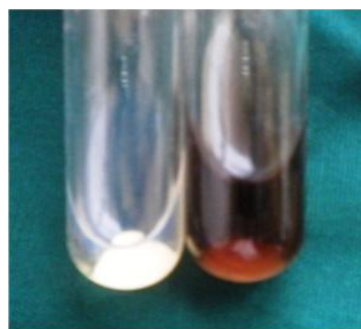
The experiments were conducted in several stages each with its own control. To compare the antimicrobial activity of the agent, the counted CFU were calculated in percentage of the respective control (mean $\pm$ SD). For all experiments, the absolute numbers of CFU, the percentage values and the means with standard deviation of the independent trials are given. Data were analyzed by one way ANOVA with Tamhane post hoc tests to compare independent samples ($\alpha=0.05$).

The effect of aqueous, gaseous ozone and 0.5% NaOCl on *Enterococcus faecalis* in planktonic culture was evaluated & calibrated. Concentrations of gaseous ozone for 1 min/ $53 \mu\text{g}$ almost and aqueous ozone completely eliminated the suspended microorganisms as did 0.5% NaOCl and no viable growth was noticed on the streaked petridish after incubation for 24 hr at 37°C .

IV. Figures And Tables



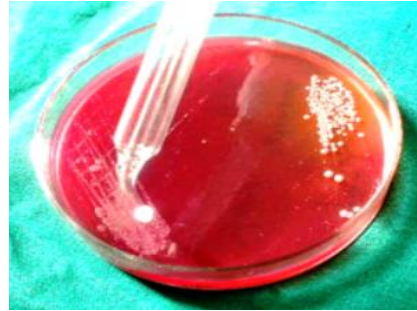
1 Mixed culture



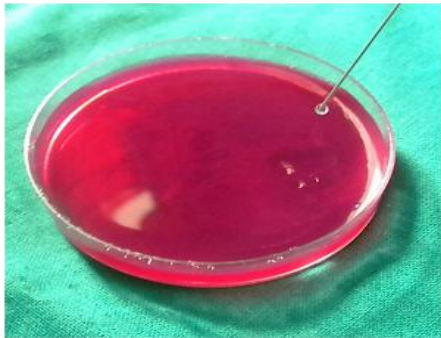
2 bile test



3. Pure culture of *E. faecalis*



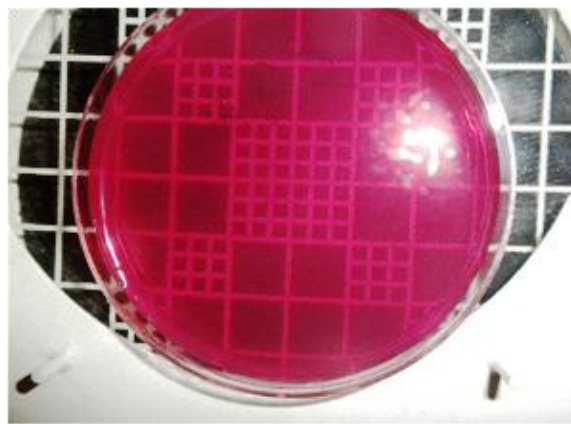
4. ozone gas



5 Sparging of ozonated water



6. CFU determined after 24 hrs (ozone gas)

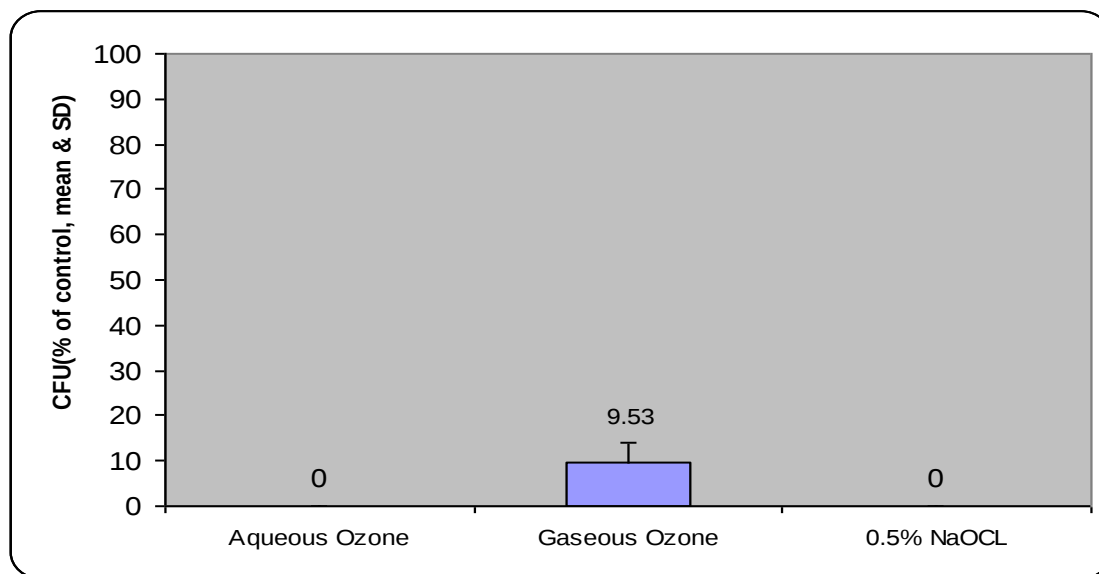


7. CFU determined after 24 hrs (ozonated water)

The antimicrobial efficacy of the solutions as irrigants is shown in the graph 1 & table 1

	Aqueous Ozone	Gaseous Ozone	0.5% NaOCL
Mean	0	9.53	0
SD	0	4.56	0

Table 1



Graph 1

V. Discussion

Factors that may contribute to a persistent periradicular infection after root canal treatment include intraradicular infection, extraradicular infection, foreign body reaction, and cysts containing cholesterol crystals. It is generally believed that the major cause of failure is the survival of microorganisms in the apical portion of the root-filled tooth

Sundqvist et al.⁹ 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*, a Gram-positive facultative anaerobe, was chosen as the test microorganism because it has significant implication in treatment resistant cases, and is difficult to kill. *E. faecalis* is able to survive in the adverse conditions as a virtue of certain biological processes and contains certain virulence factors including lytic enzymes, cytolysin, aggregation substance, pheromones, and lipoteichoic acid. *E. faecalis* bind to type I collagen. *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 noninstrumented surfaces¹⁰. Traditionally used NaOCl is a potent antibacterial agent, the active principle of NaOCl is the amount of undissociated HOCl molecules, which are consumed in the interaction with organic matter, however, its action does not affect inorganic material¹¹, it is toxic at high concentrations (Spangberg et al. 1973) & It also weakens dentine by reducing its flexural strength and resilience¹², therefore rendering the tooth more susceptible to deformation¹³ and also One of the practical difficulties of using sodium hypochlorite solution is due to its caustic nature, which can cause damage to adjacent soft tissues. Difficulty in handling and flow properties of the solution are factors, which contribute to this problem.¹⁴

The ozone therapy has been coming up as a new therapeutic modality. & essentially non cytotoxic to oral cells. Ozone is one of nature's most powerful oxidants, and directly oxidizes the substrate through decomposition via OH, The first site to be attacked is the bacterial membrane either through the glycoproteins or glycolipids or through certain amino acids such as typtophan Ozone disrupts enzymatic activity of bacteria by acting on the sulphhydryl groups of certain enzymes. Beyond the cell membrane and cell wall, ozone may act on the nuclear material within the cell (affect both purines and pyrimidines in nucleic acids). Primary and secondary outcome measures of the present study do correlate with gulabivala et al⁷ stating that ozone in its aqueous form is more effective than its gaseous form, as the later could be neutralized very easily.

This potential oxidative power has a great antimicrobial effect and it increases the adenosine triphosphate (ATP) synthesis that can be considered as a general improvement of the cell metabolism, which can potentize the repair process. But does not possess the tissue dissolving property. Hata et al.¹⁴ concluded that OPW irrigation by syringe is as effective as 5% NaOCl or 15% EDTA for removal of smear layer and debris.

In accordance to Nagayoshi et al. (2004), gulabivala et al (2006) & estrela et al (2008) observed that ozonated water had nearly the same antimicrobial activity as 2.5% NaOCl during irrigation. The strengths of the present study was to determine the eradication of a persistent organism by a noble method whereas the only limitation was the gaseous ozone being highly instable & was neutralized easily with no marked potency.

The effectiveness of ozone is highest in solution & gaseous ozone would not be expected to exert any great antibacterial effect. This observation reiterates the resistance of *E. faecalis* to adverse conditions.

Implying to the current evidence that a high level of biocompatibility of aqueous ozone on human oral epithelial (BHY) cells, gingival fibroblast (HGF-1) cells, and periodontal cells have been noted. But manufacturer's directions must be followed in order to prevent any potential lung inhalation, which is also reversible with time. Ozone is an effective, easy, cheap, and fast treatment to help disinfect root canals, and is much stronger than chlorine and acts 3,000 times faster without producing harmful decomposition products.

VI. Conclusion:

High-concentrated gaseous and aqueous ozone was dose, strain- and time-dependently effective against the tested microorganisms in suspension. The effectiveness of ozone is highest in solution & gaseous ozone would not be expected to exert any great antibacterial effect. This observation reiterates the resistance of *E. faecalis* to adverse conditions. Implying to the current evidence that a high level of biocompatibility of aqueous ozone on human oral epithelial (BHY) cells, gingival fibroblast (HGF-1) cells, and periodontal cells have been noted. But manufacturer's directions must be followed in order to prevent any potential lung inhalation, which is also reversible with time. Ozone is an effective, easy, cheap, and fast treatment to help disinfect root canals, and is much stronger than chlorine and acts 3,000 times faster without producing harmful decomposition products.

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