

Which is the most effective disinfection method in primary root canals: Conventional or newly developed ones?

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Abstract

Background: The aim of the study was to evaluate the antimicrobial activity of the-potassium-titanyl-phosphate - the KTP laser and ozone in of primary root canals.

Materials and Methods: Sixty primary incisor teeth were selected. The specimens were inoculated with 10 µL *Enterococcus faecalis* (*E. faecalis*). Groups: The KTP laser (1.5 W); gaseous ozone (150 s); sodium hypochlorite (NaOCl); saline group. Sterile paper points used to sample bacteria from the canals to tubes containing 5 mL of brain heart infusion broth. Then, 10 µL suspension was incubated in culture media for 24 h. Data were analyzed statistically using Kruskal-Wallis and Mann-Whitney U-test.

Results: There were statistically significant differences between all groups ($P < 0.05$). Complete sterilization was achieved in the 2.5% NaOCl group. The number of bacteria were significantly reduced in experimental groups in comparison to the saline group.

Conclusion: The KTP laser and ozone application provided a significant antibacterial effect in primary root canals; however, 2.5% NaOCl was superior.

Key words: Antibacterial activity, KTP laser, ozone, primary root canals

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Introduction

Teeth with infected root canals, particularly those in which the infection has reached the periradicular tissues, are a common problem in the primary dentition. Early loss of primary teeth can cause a number of problems, including space loss for successor permanent teeth, esthetic, phonetic or functional problems. However, some of the infected primary teeth can remain functional until the exfoliation via endodontic treatment. Pulpectomy is indicated in a primary tooth with irreversible pulpitis in which the radicular pulp exhibits clinical signs of pulp necrosis or shows evidence of chronic inflammation in the radicular pulp.^[1]

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The primary objective of pulp therapy is to maintain the integrity and health of the teeth and their supporting tissues.^[1] One of the most important aims of endodontic treatment is to eradicate or substantially reduce the microbial load in the root canal system. Approximately, 150 species of microorganisms are able to colonize in root canals, and some of them are responsible for lesions associated with pulpal necrosis. These microorganisms are also found in the dentinal tubules and may reinfect the root canal if they are not eliminated. Among the microorganisms commonly isolated from root canals, *E. faecalis* demonstrates the

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important abilities of penetrating the dentinal tubules,^[2] exhibiting strong adhesion to collagen,^[3] and showing resistance to irrigation solutions usually used during the instrumentation of root canals.

An ideal irrigation solution should be strongly antimicrobial but not toxic to the periradicular tissues when extruded through the apical foramen. Sodium hypochlorite (NaOCl) is by far the most commonly used irrigant in endodontic treatment. It provides gross debridement, lubrication, the destruction of microbes, and the dissolution of tissues. In addition, it is inexpensive, has a long shelf life, and is readily available. However, this irrigant leads to problems including toxicity, odor, and the discoloration of operatory items. Additionally, its extrusion can cause excruciating pain, immediate swelling, and profuse bleeding. It may also result in allergic reactions.^[4] Therefore, an alternative endodontic antiseptic with high antimicrobial potential and fewer side effects would be valuable.

Ozone is an extremely reactive gas that shows important antimicrobial properties. Its natural form is found in the atmosphere, or it can be produced by generators. This gas oxidizes bacterial cell walls and cytoplasmic membranes and acts on fungi, protozoa, and viruses. It forms oxidized radicals in the presence of water that penetrate and act on cell membranes, affecting the osmotic stability, promoting the oxidation of aminoacids and causing cellular lysis depending on the reaction's extension.^[5] Ozone has also been used in the water industry to eliminate bacteria, and its properties could be useful in dentistry for the elimination of caries pathogens, in the disinfection of root canals, during surgery, and as a rinse for avulsed teeth, for failed implant cases.^[6,7]

Laser irradiation has been introduced due to its potential to eliminate bacteria and thus improve endodontic treatment. Thereafter, antimicrobial effect of different types of laser irradiation on root canals has been evaluated. These types are the carbon dioxide;^[8] neodymium-doped yttrium aluminum garnet (Nd: YAG);^[9] Erbium, Chromium doped Yttrium Scandium Gallium Garnet;^[10] Erbium doped Yttrium Aluminum Garnet;^[11] KTP;^[12] and Diode lasers.^[13] Lasers have been found to be relatively effective in exerting antimicrobial action;^[9-11,13] the bacterial reduction has depended on radiation energy, bacterial species, time of radiation,^[11] and radiation frequency.^[9] The KTP laser emitting at 532 nm and representative of a frequency-doubled Nd: YAG device, has been introduced mainly for tooth-bleaching procedures in dentistry. It can be delivered through a wide range of fibers in a constant or a pulsed mode.^[14] This laser has also been used for some other dental applications similar to Nd: YAG laser, including root canal disinfection, treatment of dentine hypersensitivity, and soft tissue surgery.

To our knowledge, the antimicrobial effect of KTP laser and gaseous ozone in primary teeth has not yet been studied. Therefore, the purpose of this *in vitro* study was to compare the antibacterial effect of KTP laser irradiation and gaseous ozone in primary root canals contaminated by *E. faecalis*.

Materials and Methods

Sixty freshly extracted, single-rooted human primary teeth with straight root canals were selected in this study. Teeth were extracted for infection, extensive caries (for which parents choose extraction rather than pulp therapy for primary teeth) or trauma and did not have any radiographically visible physiological or pathological root resorption. All patients and parents whose extracted teeth were used in the study gave written consent for this to happen.

Sample size calculation

Sample size was calculated using a sample size calculator (Sample Size Determination in Health Studies, World Health Organization) as follows. Power at 90% and α at 5%, β at 10%, the sample size turned out to be 15 teeth in each group. Thus, a total of 60 extracted single-rooted primary teeth was required for the study.

Tooth preparation

Teeth were immersed in 5.25% NaOCl for 15 min to remove organic residues from root surfaces. Stains and calculus were removed with scalers and curettes. Care was taken to ensure that the teeth were not subjected to any treatment before their extraction, and they were stored in a sterile saline solution at +4°C until the experiment. Crowns of the teeth were reduced to the cemento-enamel junction. An access cavity was prepared, and the pulp was removed with a barbed broach.

Working length (WL) of each canal was determined using a size 15 K-file (Kerr-files; Maillefer, Ballaigues, Switzerland). The file was placed in the canal until its tip became visible from the apical foramen. WL was determined to be 1 mm shorter than this length. Experimental model that was used for preparation is presented in Figure 1. With a hot instrument, holes were quickly created on the cap of the Eppendorf tubes. The tooth was inserted under pressure through the cap of the Eppendorf tubes, which was fixed by means of cyanoacrylate [Figure 1]. The tooth was the Eppendorf tube cap was then fitted into the mouth of the tube, and the root canal was enlarged using K-files up to a size 40. 2 ml of 9% saline solution was used as an irrigant, and the canals were dried using paper points. At the end of instrumentation, smear layer formed in the canal walls during the instrumentation was removed with 17%

ethylenediaminetetraacetic acid for 3 min and then a final irrigation was accomplished with 4 mL of 0.9% saline solution.

After preparation, two coats of nail varnish were applied to the external surface of all roots to prevent bacterial microleakage through lateral canals or other discontinuities in the cementum. A hole was created in the nail varnish that covered the apical foramen using a size 15 K-file. During this procedure, only 1 mm of file was extruded. As a result of this procedure, a standard size of foramen and apical patency was achieved. The entire model system was then sterilized in ethylene oxide gas for a 12-h cycle.

Contamination of root canals

A total sum of 60 root canals was inoculated with *E. faecalis* (ATCC 29212) grown in brain heart infusion (BHI) broth (Difco, Detroit, MI, USA). The McFarland standard number of 0.5 was used to evaluate the broth to ensure that the number of bacteria was 1.5×10^8 colony-forming units (CFU) mL^{-1} . A 10- μL amount of the bacterial culture was transferred into the canal lumen of the mechanically enlarged root canals using a sterile micropipette, and it was then stored for 24 h at 37°C.

Experimental design

The contaminated roots were divided into 2 experimental, 1 negative control, and 1 positive control groups each including 15 teeth.

Group 1: Ozone group

Gaseous ozone was applied with an ozone generator (HealOzone, KaVo, Biberach, Germany) with a 4 g m^{-3} ozone concentration was activated to dried root canals for 150 s using a hand piece, silicone caps (for sealing), and endodontic cannulas [Figure 1].



Figure 1: The experimental model system and applying gaseous ozone

Group 2: Laser group

Canals were irradiated at 1.5 W, 10 J/cm² with the KTP laser (SMARTLITE D, Deka, Calenzano Firenze, Italy). Throughout laser treatment, the fiber tip (diameter of 200 μm) was applied with a spiral movement starting 1 mm short of the apex and then moving coronally for 5 s, interleaved with 15-s recovery intervals for each irradiation. This process was repeated 5 times [Figure 2].

Group 3: Negative control group

Canals were irrigated with 2 mL of 2.5% NaOCl using a plastic syringe with 27 gauge needle. The pressure applied by the operator on the syringe sufficiently allowed for a flow rate of 2 mL of irrigant per minute. Ten serial rinses were done with a 10 min contact period between bacteria and irrigant.

Group 4: Positive control group

Canals were irrigated with 2 mL of 0.9% sterile saline, and these served as controls.

Bacterial evaluation

Before the disinfection procedures, canals in all the groups were rinsed with 1 mL of 0.9% sterile saline solution. Salin solution was collected from canals with size 40 sterile paper points for a standard 1 min contact for the sample collection. Paper points were then transferred to Eppendorf tubes containing 5 mL of BHI broth. All collected samples were vortexed for 5 min, and 10 μL of suspension was inoculated onto 1 part of duplicated blood agar plates, which were divided into two. Initial mean number of viable microorganisms was determined 5×10^5 CFU mL^{-1} ($5.477 \log \text{CFU mL}^{-1}$) for all teeth. After treatment, experimental canals of all the groups were rinsed with 1 mL of 0.9% sterile saline solution. Thereafter sterile size 40 paper points were left in the root canals for 1 min and transferred to Eppendorf tubes containing 5 mL of BHI broth. Tubes were vortexed for 5 min, and 10 μL of suspension was inoculated onto

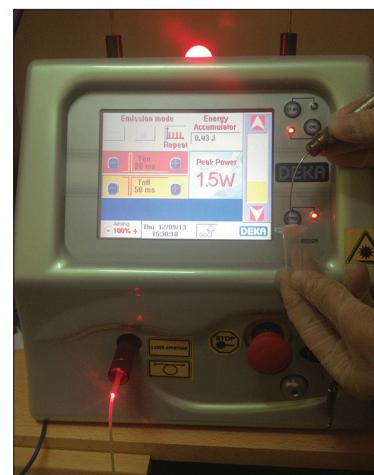


Figure 2: KTP laser device

second part of duplicated blood agar plates. The blood agar plates were incubated for 24 h at 37°C; and CFU mL⁻¹ were enumerated per root canal sample. Colonies of bacteria were counted, and the results were given as the number of log CFU mL⁻¹.

Statistical analysis

Statistical tests were performed using SPSS (Version 15.0; SPSS Inc, Chicago, IL, USA). Data were analyzed statistically using Kruskal-Wallis, and Mann-Whitney U-test. The level of statistical significance was set at $P < 0.05$.

Results

The results, reported in Table 1, show that the most effective group with complete sterilization in all the samples was Negative Control Group (2.5% NaOCl). However, the least effective group was Positive Control Group (0.9% saline). Although KTP laser irradiation and gaseous ozone provided in a significant reduction of the *E. faecalis* bacterial load, neither could achieve complete inhibition of bacteria. There was statistically significant differences between the KTP laser group and gaseous ozone group, the KTP laser group and control groups, the gaseous ozone group and control groups, and the positive and negative control groups ($P < 0.05$).

Discussion

In this study, *E. faecalis*, a Gram-positive facultative anaerobic coccus, which is a well-known endodontic pathogen, was selected for the infection of the root canals. It has been frequently recovered from root canals associated with posttreatment diseases and persistent apical periodontitis. It was also reported to be resistant to intracanal medicaments such as calcium hydroxide.^[15] In a polymerase chain reaction-based *in vivo* study, Cogulu *et al.*^[16] determined that *E. faecalis* and *Treponema denticola* were highly associated with periapical radiolucency and previous pain in both primary and permanent teeth.

One of the aims of root canal treatment is to eliminate the bacteria, their products, and the substrate from the root canal system. The use of an irrigation solution in this

process is essential to ensure bacterial elimination and the digestion of organic tissue remnants. The antimicrobial efficacy of NaOCl against *E. faecalis* in permanent teeth has been evaluated and documented in the dental literature.^[12,17] Eldeniz *et al.*^[10] showed that 3% NaOCl inhibited the growth of *E. faecalis* and provided complete elimination of the bacteria in all canals. Vianna *et al.*^[18] and Kustarci *et al.*^[12] showed that 2.5% NaOCl kills *E. faecalis* in 10 min. Approximately, 2.5% NaOCl showed a similar antibacterial effect in the present study. The antimicrobial effectiveness of NaOCl in root canals is reported to be a function of concentration and contact time.^[19] Nevertheless, Siqueira *et al.*^[19] suggested that low concentrations of NaOCl may significantly reduce the endodontic infection but might not consistently dissolve all remnants in a reasonable time; as a result, the efficacy of weak solutions may decrease rapidly. A low concentration NaOCl (2.5%) solution was selected for the disinfection of primary root canals, and a contact time of 10 min was administered in this study in order to achieve comparable results with the KTP laser and gaseous ozone evaluated.

The use of ozone has been justified as a new option of irrigating agent with antimicrobial action. The antimicrobial effect of ozone results from oxidation of microbial cellular components. Ozone is a highly reactive form of oxygen that is generated by passing oxygen through high-voltage. In a recent study, *E. faecalis* biofilms were grown over 60 days in root canals and applied ozonated water, ozone gas, NaOCl 2.5% or CHX 2% for 20 min.^[20] In contrast to the present results, none of the irrigants were found to have an antimicrobial effect.

In the present study, there were significant reductions of *E. faecalis* in the ozone group. This antibacterial effect of the gaseous ozone can be explained by the methodologies used that the gaseous ozone was applied for 150 s to root canals. This duration can be adequate, and it is also possible that the ozone gas applied into the moist root canals, as currently performed with the HealOzone device, dissolves in the root canals. As a result, an aqueous ozone formed, which then became more effective to the bacteria.^[12]

Stoll *et al.*^[21] determined the antibacterial effect of gaseous ozone obtained from a HealOzone generator during 120 s in root canals inoculated with *E. faecalis*. Additionally, researchers reported the beneficial effects of gaseous ozone for disinfecting root canal systems, in the case of NaOCl, could not reach deep enough into the root canals. Kustarci *et al.*^[12] examined the antimicrobial effects of 120 s gaseous ozone in root canals infected by *E. faecalis*. As a result, they found a reduction of the *E. faecalis* after the application of gaseous ozone.

Table 1: Mean numbers of remaining bacteria (CFU/mL)

Groups	Total (n)	Mean ± (SD) (CFU/mL)
Gaseous ozone ^{a,b,c,d}	15	2.133 ± (1.960)
KTP laser ^{b-a,c,d}	15	6.967 ± (3.765)
NaOCl (negative control) ^{a,b,d}	15	0
Saline (positive control) ^{d-a,b,c}	15	124.000 ± (72.295)

^aGaseous ozone; ^bKTP laser; ^cNaOCl; ^dSalin; $P < 0.05$. CFU=Colony-forming unit; SD=Standard deviation; KTP=Potassium-titanyl-phosphate; NaOCl=Sodium hypochlorite

Huth *et al.*^[17] investigated the effectiveness of gaseous ozone against microorganisms in a root canal biofilm model, and they reported that the total elimination of the microorganisms, in terms of the methods used in their study, could be achieved by ozone gas at 32 g m^{-3} for 1 min or by a lower concentration (4 g m^{-3}) for longer contact times ($\geq 2.5 \text{ min}$) in case of *E. faecalis*. The duration of action was, therefore, an important consideration in its antibacterial effect.

Case *et al.*^[22] examined the effects of gaseous ozone delivered into saline on biofilms of *E. faecalis* in root canals of extracted teeth, with and without the use of passive ultrasonic agitation. They reported that although none of the treatment regimes were able to reliably render canals sterile under the conditions used, ozone gas delivered into irrigating fluids in the root canal may be useful as an adjunct for endodontic disinfection. Halbauer *et al.*^[23] evaluated the effect of ozone gas on the remaining bacteria after the chemomechanical instrumentation of tooth root canal. The results of their study showed the efficacy of ozone on the bacterial count reduction in the root canal treatment.

The antimicrobial efficacy of various lasers against *E. faecalis* in permanent teeth has been evaluated and documented in the dental literature.^[24] It was suggested that, besides the improved removal of debris and smear layer, dental lasers could provide greater accessibility to formerly unreachable parts of the tubular network because of their enhanced penetration into dentinal tissues;^[25] consequently they may have ancillary antimicrobial effects to aid in the reduction of bacteria in the root canal.^[26]

In this study, the KTP laser was used in the primary root canals for disinfection. Few reports on the use of KTP lasers have been published. Tewfik *et al.*^[27] reported that when used within the canals, this laser did not modify the permeability of the smear layer-covered dentine. However, they noted that SEM examination revealed modifications to the surface of the smear layer with no subsequent effects on the underlying dentine. When used on etched dentine, lasing produced a modest increase in the root permeability associated with the enlargement and cracking of tubule orifices.

Kanamaru *et al.*^[28] focused on the influence of the KTP laser on the root canal surface and on the extent of the temperature increase at the root surface *in vitro*. They found that KTP laser irradiation facilitated the removal of the smear layer from the root canal surface. Thermography revealed the harmlessness of the procedure in reference to the temperature rise on the root surface.

Also, only a few studies have evaluated the antibacterial effect of the KTP laser. Schoop *et al.*^[14] studied the

antibacterial effect of the KTP laser on the root canal dentin, and they found a reduction of the *E. faecalis* and *Escherichia coli*. Simsek *et al.*^[29] examined the bacterial microléakage of the root canals irrigated with different irrigation solutions and the KTP laser system and filled with Gutta-percha and AH26 root canal sealer. Researchers showed that different irrigation solutions and the KTP laser allowed microléakage of *E. faecalis*.

Kustarcı *et al.*^[12] determined the antimicrobial activity of KTP laser and gaseous ozone in experimentally infected permanent root canals. As a result, they found a reduction of the *E. faecalis* after application of KTP laser. Our results were similar to these previous studies.

In a previous study, Schoop *et al.*^[14] suggested that the protocol of lasing 5 times for 5 s, followed by a resting time of 15 s for the samples, irradiated at 1.5 W for both laser systems. These authors reported that the KTP laser protocol tested in that study may be a suitable tool for the disinfection of canals and can be safely applied if the common precautions are observed and the applied energy stays within the proposed range. Therefore, in the present study, the KTP laser was used in pulse mode, 1.5 W for 5 s, 5 times for each root canal with 15 s cooling time between each irradiation with the fiber tip (diameter of 200 μm).

In this study, there was significant bacterial reduction in the laser group. We thought that directing the laser beam onto the canal wall with 200- μm diameter endodontic laser fiber, which enables better direction of the laser light into the root canals. Also, given the characteristics of laser light and the fact that direct contact between target and fiber tip is not required, the emission of laser energy could represent a way to disinfect areas deep within the dentine. When laser energy is absorbed by the target, a reaction may occur depending on the total amount of energy applied, whereas the interaction type depends on power density (W cm^2) and pulse duration. A photo-thermal interaction with bacteria will represent a bactericidal effect.^[30] However, in the present study, gaseous ozone group showed more of an antibacterial effect than the KTP laser did. This difference might be due to the fact that gaseous ozone, when compared to the KTP laser, can more effectively permeate the entire surface of the root canals and into dentinal tubules. Because the laser beam delivers its energy only where it is focused, it may not be possible to direct the beam over the entire surface of the root canal wall.^[12]

Under *in vivo* conditions, the microflora of an infected root canal consists of multiple types of microorganisms that may have synergistic interactions with each other. However, it is practically impossible to duplicate this clinical environment in an *in vitro* study such as the present one. Though the utilization of only one type of microorganism

can be considered as a drawback in the present study, it can be speculated that a reliable comparison could still be performed regarding the influence of different disinfection protocols. Although care was taken to standardize the assessment conditions of the three disinfection methods evaluated in this study, due to the difference in the mechanisms of action for each group, some variations with regard to the technique are always expected.

KTP laser and gaseous ozone application provided a significant antibacterial effect in primary root canals. However, NaOCl was superior in its antimicrobial abilities compared to the KTP laser and gaseous ozone. Gaseous ozone, in comparison to the KTP laser, was found to be more effective in reducing the number of bacteria. However, both the KTP laser and gaseous ozone were insufficient in eradicating all bacteria.

This present study showed that laser irradiation and gaseous ozone could be aids to NaOCl in endodontic treatment, and they should be used at the end of instrumentation. The results of the study should be confirmed with clinical investigations in order to suggest laser irradiations and ozone application for the disinfection of root canals of primary teeth.

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