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Bactericidal efficacy of Er,Cr:YSGG laser irradiation against *Enterococcus faecalis* compared with NaOCl irrigation: an *ex vivo* pilot study

A. U. Eldeniz[1], F. Ozer[2], H. H. Hadimli[3] & O. Erganis[3]

[1]Department of Endodontics; [2]Department of Operative Dentistry, Faculty of Dentistry; and [3]Department of Microbiology, Faculty of Veterinary Medicine, University of Selcuk, Konya, Turkey

Abstract

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Aim To compare the efficacy of a standard NaOCl irrigation procedure with that of Er,Cr:YSGG laser irradiation in contaminated root canals having small and large apical foramina.

Methodology Forty root canals of extracted central incisor teeth with straight roots were chosen so that their apical foramina just permitted the tip of a size 20-K file to pass through. The canals were then enlarged with files to size 60 and randomly divided into four groups of 10 teeth each. The apical foramina of one group were widened further so that the tip of a size 45-K file could just pass through. After sterilization, all roots were inoculated with *Enterococcus faecalis* for 48 h at 37 °C. The first group was used as a control, the second group was irrigated with 3% NaOCl solution for 15 min, and the last two groups having different sizes of apical foramina were irradiated with the Er,Cr:YSGG laser at output power from 0.5 W, with 20% air and water levels. The disinfecting efficacy of the groups was tested by collecting dentine chips from the inner canal walls of the specimens and counting viable *E. faecalis* on Mueller–Hinton agar plates.

Results The differences in the mean number of viable colonies between the control and laser groups were statistically significant ($P < 0.05$). The control specimens had the highest number of microorganisms ($153 \times 10^3 \pm 39 \times 10^3$). Complete sterilization was achieved in the 3% NaOCl group. The mean colony forming units (CFU) values obtained after Er,Cr:YSGG laser irradiation were 6.6×10^3 CFU and 6.5×10^3 CFU in root canals having large and small apical foramina respectively.

Conclusion In teeth with straight roots the Er,Cr:YSGG laser reduced the viable microbial population in root canals with small and large apical foramina but did not eradicate all bacteria. Three percent NaOCl inhibited the growth of *E. faecalis* and effectively sterilized all root canals.

Keywords: *E. faecalis*, Er,Cr:YSGG laser, root canals.

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Introduction

Obtaining a root canal system free of irritants is a major goal of root canal treatment because remaining microorganisms may cause persistent inflammation in the periradicular tissues (Molander *et al.* 1998). In addition to the mechanical instrumentation of the root canal system, irrigating the canal with disinfecting chemicals (Jeansonne & White 1994) has been proposed to enhance the removal of vital and nonvital tissue remnants, tissue breakdown products, bacteria and bacterial byproducts (Byström 1986). However, the existence of accessory canals, anastomoses and fins

Correspondence: Ayce Unverdi Eldeniz, Department of Endodontics, Faculty of Dentistry, Selcuk University, Konya, Turkey (Tel.: +90 332 241 00 41; fax: +90 332 241 00 62; e-mail: aunverdi@selcuk.edu.tr).

creates a three-dimensional network that makes the complete elimination of debris and achievement of a sterile root canal system difficult with conventional irrigation solutions (Byström & Sundqvist 1981). The apical third of the instrumented root canal remains partially untreated with insufficient removal of debris and necrotic soft tissue (Haikel & Allemann 1988). In addition, in mechanically treated root canals, a smear layer is created consisting of dentine debris, pulpal cells and bacteria (Drake *et al.* 1994). The smear layer may interfere with the effectiveness of irrigation solutions in the root canal (Ørstavik & Haapasalo 1990) because of insufficient penetration within the deeper layers of the dentine (Berutti *et al.* 1997).

Laser radiation has the potential to aid in endodontic treatment (Stabholz *et al.* 2004). 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 (Vaarkamp *et al.* 1995, Klinke *et al.* 1997) and consequently may have ancillary antimicrobial effects to aid in the reduction of bacteria in the root canal (Hardee *et al.* 1994, Moshonov *et al.* 1995, Mehl *et al.* 1999).

The disinfecting ability of different types of lasers in laboratory-based models has been studied from several aspects using the Er:YAG (Jelínková *et al.* 1999, Mehl *et al.* 1999, Moritz *et al.* 1999, Dostálová *et al.* 2002, Schoop *et al.* 2002, Schoop *et al.* 2004), the Nd:YAG (Levy 1992, Hardee *et al.* 1994, Rooney *et al.* 1994, Fegan & Steiman 1995, Moshonov *et al.* 1995, Gutknecht *et al.* 1996, Ramsköld *et al.* 1997, Moritz *et al.* 2000, Folwaczny *et al.* 2002, Piccolomini *et al.* 2002, D'Ercole *et al.* 2004, Schoop *et al.* 2004), the Ho:YAG (Gutknecht *et al.* 1997, Moritz *et al.* 1999), the CO_2 (Zakariasen *et al.* 1986, Dederich *et al.* 1990, Le Goff *et al.* 1999), the Excimer (Stabholz *et al.* 1993, Folwaczny *et al.* 1998), the Nd:YAP (Blum *et al.* 1997), the Diode (Gutknecht *et al.* 2000, 2004) and the Alexandrite (Jelínková *et al.* 1999) systems. All lasers were found to have variable bactericidal effects.

Different lasers react with dental tissues in different ways, depending on their 'absorption coefficient'. Lasers which are well absorbed in water and hydroxyapatite are considered suitable for hard tissue lasing. The first laser considered suitable for hard tissue applications was the Er:YAG laser (2.94 μm). As a result of advances in the field of laser-assisted endodontics a new hard tissue laser, the erbium, chromium: yttrium-scandium-gallium-garnet (Er,Cr:YSGG) laser at 2.78 μm has become available. This laser uses specialized thin and flexible endodontic fibre tips of various diameters and lengths that provide easy access to pulpal tissues and tooth structure and prepare the canal for filling. Although thermal injury to periodontal tissues is of concern during laser usage for intracanal applications, it was reported that the temperature rise during Er,Cr:YSGG laser irradiation is minimal and does not cause damage to periradicular bone and tissues (Ishizaki *et al.* 2004). In addition, its effectiveness at removing smear layer and debris has been demonstrated (Yamazaki *et al.* 2001, Ishizaki *et al.* 2004). The Er,Cr:YSGG laser system uses hydrokinetic energy – the laser energy heats the air and water directly in front of the laser beam to deliver energy onto the rear surface of atomized water molecules with the aim of accelerating them to a higher speed. As a result of this hydrokinetic energy, the Er,Cr:YSGG laser may have a greater ability to disinfect root canals.

There are cases in clinical practice where the apical foramen is large because of over instrumentation, root-end resection during periradicular surgery, incompletely formed root apex or inflammatory root resorption of endodontic origin (Kerezoudis *et al.* 1999, Vier & Figueiredo 2004). With laser disinfection, most of the energy from the light-conducting fibre is emitted in an axial direction and not toward the canal wall. Consequently, the effect of the laser beam may vary when teeth have different sizes of foramina.

The antibacterial efficacy of Er,Cr:YSGG laser irradiation in infected root canals in comparison with sodium hypochlorite irrigation has not been evaluated. Furthermore, the influence of the size of apical foramina on the bactericidal effectiveness of this laser device in root canals is unknown.

The aim of the present pilot study was to compare the efficacy of a standard irrigation procedure with that of the Er,Cr:YSGG laser technique in contaminated root canals and also to test the Er,Cr:YSGG laser system in contaminated root canals with two different sizes of apical foramina.

Materials and methods

Forty extracted central incisor teeth with similar dimensions, completely formed apices and straight roots were selected. The teeth had been extracted for periodontal reasons and were scaled and cleaned of debris and periodontal remnants. The teeth were chosen so that their apical foramina just permitted the tip of a size 20-K file to pass through. The crowns

were removed at the cemento-enamel junction to obtain a root canal length of 12 mm and a size 10-K file was extended just beyond the apical foramen to ensure that the canal was patent before further instrumentation. The working length of each root canal was established 1 mm short of the apical foramen with a size 15 reamer (GC, Tokyo, Japan). Gates Glidden burs sizes 2, 3 and 4 were used to flare the coronal 1/3 of each canal. Canals were instrumented with hand stainless steel instruments to size 60 (K-type file; Mani Inc., Nakaakutsu, Japan) using physiological saline as an irrigant. These teeth were then randomly assigned to one of four groups with 10 roots each: the control group, the NaOCl group and two laser groups. The apical foramina of one of the laser groups was then further widened until the tip of a size 45-K file just passed through with minimal pressure. The smear layer was removed by the sequential use of 17% EDTA and 5.25% NaOCl, for 3 min each. Sterility of the groups was achieved using an autoclave at 121 °C for 15 min with the teeth immersed in distilled water. To confirm sterility, the teeth were placed in brain heart infusion broth (Oxoid, Basingstoke, UK) and incubated for 24 h at 37 °C.

Selection and preparation of bacteria

A pure bacterial culture of the Gram-positive *cocci*, *Enterococcus faecalis* (ATCC 10541) was obtained from the Culture Collection (RSHSE, Ankara, Turkey). Before starting the experiments, the frozen (−20 °C) bacterial sample was thawed and incubated for 24 h on a solid culture medium (Brain Heart Infusion Agar, supplemented with 7% sheep blood; Oxoid Ltd., Basingtoke, UK) at 37 °C under aerobic conditions. The grown bacterial colonies were then harvested, placed in Mueller–Hinton nutrient broth (Difco Laboratories, Detroit, MI, USA) and incubated for an additional 24 h at 37 °C under aerobic conditions. The *E. faecalis* culture was then calibrated to 2.5×10^8 colony forming units per mL (CFU mL^{-1}) spectrophotometrically in Mueller–Hinton broth and 20 μL of the bacterial culture were transferred into the canal lumen of the mechanically enlarged root canals using a sterile micropipette (Eppendorf, Hamburg, Germany) and then stored for 48 h at 37 °C.

Canal treatment

After 48 h, all canals were dried with sterile paper points. The first group containing canals with an apical size of 0.2 mm was used as a control and received no treatment (control group). The second group containing canals with an apical size of 0.2 mm was treated with a 15 mL of 3% NaOCl solution (Çağlayan Kimya, Konya, Turkey) by delivering the solution 1 mm from the working length using sterile 5.0 mL plastic syringes and 21-gauge needles (Sterijen; Hayat Tıbbi Aletleri, Istanbul, Turkey) (NaOCl group). Three serial rinses ensured a standard 15 min contact period between bacteria and antiseptic solution. The root canals of groups three and four were irradiated with the Er,Cr:YSGG laser (Waterlase Millennium; Biolase Tech., San Clemente, CA, USA) emitting a wavelength of 2,780 nm. In this device, pulse energy can be varied between 25 and 300 mJ at a fixed repetition rate of 20 Hz. This results in an output power of 0.5–6 W. In this study, output power was 0.5 W, with 20% air and water levels as recommended by the manufacturer for root canal sterilization. A fibre tip with a diameter of 200 μm was used (Milennium; Biolase Technology Inc. (P/N 5000602)). The fibre tip was inserted into the root canal at a distance of 1 mm from the apical foramen and moved in two consecutive cycles from apex to crown at a constant speed of approximately 1.5 mm s^{-1}. The difference between the two laser treated groups was the size of the apical foramina: one group had an apical foramen size of 0.2 mm (small apical foramen group) whereas the other had an apical foramen size of 0.45 mm (large apical foramen group).

All 40 teeth were kept in a freezer at −25 °C for 1 h for cooling before sampling to avoid killing *E. faecalis* because of heat from the drills during sampling. Disinfecting efficacy was determined by collecting dentine chips with Gates Glidden burs (Mani Inc.), sizes 5 and 6 from the inner canal walls of the specimens. The drills were applied 1 mm short of the apex, just above the apical foramen without extending to the apical foramen area and 10 mg dentine chips from each canal were obtained and placed onto plates which were weighed before and during dentine collection on an electronic balance (Electronic Balance Type AX200; Shimadzu Corporation, Kyoto, Japan). The samples were then transferred to test tubes containing 2 mL of sterile physiologic saline, vortexed for 20 s and serial 10-fold dilutions were prepared to a concentration of 10^{-7}. Then, 100 μL of each dilution was applied to three Mueller–Hinton agar culture plates and incubated at 37 °C for 48 h. All procedures were conducted inside a laminar flow chamber using sterile instruments to achieve strict asepsis. A classical bacterial counting technique was used for recovery of

viable *E. faecalis* on Mueller–Hinton agar plates (Collins *et al.* 1995). Bacterial growth was not obtained in the agar plates of concentration 10^{-5}, 10^{-6} and 10^{-7}. The mean number of CFU for the three plates was calculated for each of the concentrations from 10^{-2} to 10^{-4}. Resulting mean values were converted to the concentration of 10^{-3} and were added together and then divided by three to obtain one CFU value per sample.

Statistical analysis

The mean and standard deviation of CFU values were calculated. Statistical analysis was performed using the SPSS program for Windows 10.0 (SPSS Inc., Chicago, IL, USA). As the data were nonparametric, CFU values were subjected to Kruskal–Wallis test for significant differences. The Bonferroni adjusted Mann–Whitney U-test was used for group comparisons ($\alpha = 0.05$).

Results

Results are reported in Table 1. The bacterial concentration in the positive control group revealed that bacteria survived the test period confirming the efficiency of the methodology. The control group had the highest number of microorganisms ($153 \times 10^3 \pm 39 \times 10^3$ CFU). In this artificial contamination model, complete sterilization was achieved in the 3% NaOCl group as no bacteria were detected on the agar plates in any of the samples. The Er,Cr:YSGG laser did not completely sterilize the root canals in either of the two laser groups. Mean CFU values after Er,Cr:YSGG laser irradiation were 6.5×10^3 CFU and 6.6×10^3 CFU in *E. faecalis* contaminated root canals having small or large apical foramina respectively. There was no statistical difference between these two laser groups ($P > 0.05$). The differences in the mean number of viable colonies between the NaOCl and laser groups were statistically significant ($P < 0.05$).

Table 1 Bacteria recovered from *Enterococcus faecalis* infected root canals *in vitro*

Group	No. of Samples (*n*)	CFUs recovered	(%)†
Control	10	$153 \times 10^3 \pm 39 \times 10^3$*[a]	100
3% NaOCl	10	0[b]	0
Laser (small apical foramen)	10	$6.5 \times 10^3 \pm 17.8 \times 10^3$[c]	4.25
Laser (large apical foramen)	10	$6.6 \times 10^3 \pm 11.5 \times 10^3$[c]	4.31

CFUs, colony forming units.
*Groups identified by different letters denote statistical significance according to Bonferroni adjusted Mann–Whitney *U*-test ($P < 0.05$).
†Percentage relative to control group CFUs.

Discussion

In this study, the root canal system was contaminated with *E. faecalis*, which is a facultative Gram-positive anaerobic *coccus* that is a known endodontic pathogen, being frequently recovered from the root canals of teeth associated with post-treatment diseases (Molander *et al.* 1998). It is also resistant to interappointment medicaments, including calcium hydroxide (Estrela *et al.* 1999, Haapasalo *et al.* 2000) and may also reside in canals as a single species without the support of other microorganisms (Fabricius *et al.* 1982). It was also reported that this microorganism has the ability under specific conditions to infect the whole length of the tubules within 2 days (Ørstavik & Haapasalo 1990). Although some authors have conducted studies using microorganisms not found in root canals, such as *Bacillus sterothermophilus*, *Escherichia coli* and *Staphylococcus aureus* (Hardee *et al.* 1994, Ragot-Roy *et al.* 1994, Fegan & Steiman 1995), it is important to validate the bactericidal action of different disinfection methods by using a resistant microorganism such as *E. faecalis* (Ørstavik & Haapasalo 1990, Ramsköld *et al.* 1997, Siqueira & de Uzeda 1997, Tanriverdi *et al.* 1997, Komorowski *et al.* 2000, Schoop *et al.* 2002). Although the ATCC strain of *E. faecalis* has been previously used in several *ex vivo* studies to test the antimicrobial action of intracanal medication and lasers (Ørstavik & Haapasalo 1990, Siqueira & de Uzeda 1997, Moritz *et al.* 2000, Schoop *et al.* 2004), in the present study *E. faecalis* cells were incubated with teeth for 48 h to use cells in the starvation phase rather than using growing cells. This was done to better simulate *in vivo* conditions which would have a limited amount of nutrients available in the root canal (Portenier *et al.* 2005).

In an attempt to reproduce bacterial root canal colonization, the methodology of Ramsköld *et al.* (1997) and Le Goff *et al.* (1999) was used to contaminate the entire root canal system. During the experiments, the *E. faecalis* strains remained in the root canals for 48 h, grew and were also able to contaminate the tubules as was confirmed by scanning electron microscope (SEM, Philips XL30 ESEM, Eindhoven, The Netherlands) and shown in Fig. 1. This protocol probably avoided a simple infection (Moshonov *et al.* 1995) which is likely to be easier to remove and is far from the true physiopathological conditions.

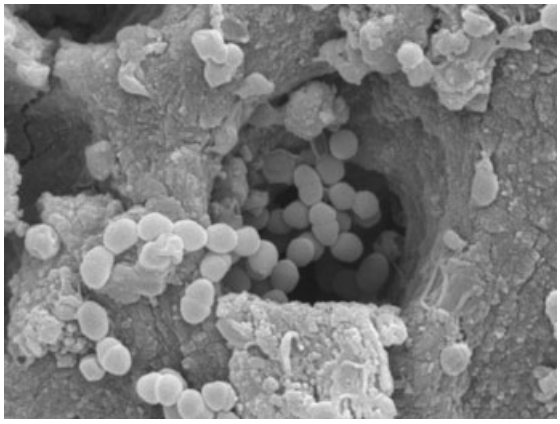

Figure 1 Contamination of the human dentinal tubules after a 48-h infection period (magnification ×10000).

Sodium hypochlorite solution was applied to the root canals for 15 min in this study because this period corresponds approximately to the time required for the biomechanical preparation of a root canal of average difficulty. The Er,Cr:YSGG laser was used according to the manufacturer's instructions for sterilization. The time of use which is equal to approximately 20 s in contaminated root canals, is advisable as the use of lasers for longer periods has a risk of creating thermal damage to dental tissues. The time difference between laser and sodium hypochlorite treatments in this study is acknowledged but reflects clinical conditions rather than the adherence to strict standardization.

In the present study the effect of NaOCl irrigation was tested in canals with small apical foramina. In a previous pilot study, it was found that NaOCl irrigation for 15 min was capable of completely sterilizing the root canals of teeth with both small and large foramina. Thus, it was considered unnecessary to test and add a group of teeth with large apical foramen in the NaOCl irrigation group.

A substantial number of central incisor teeth were collected and evaluated for this study. As large numbers of teeth had an apical foramen size of 0.2 mm, this size was chosen as a typical apical foramen size for the small apical foramen group for the evaluation of Er:Cr;YSGG laser irradiation.

In previous studies, bacterial samples were removed using paper points (Moshonov *et al.* 1995, Ramsköld *et al.* 1997), by rinsing (Hardee *et al.* 1994, Fegan & Steiman 1995, Folwaczny *et al.* 2002, Schoop *et al.* 2002), or by immersion in culture broth (Zakariasen *et al.* 1986) or physiological saline solution (Moritz *et al.* 2000, Schoop *et al.* 2004). However, using these techniques, the volumes recovered could not be quantified. In this study, the sampling procedure involving the scraping of the canal walls was used on the basis that Ørstavik & Haapasalo (1990) reported an overall good correlation between histology and culturing of dentine dust in their *in vitro* study. Peters *et al.* (2001) also confirmed that grinding and culturing of dentine gave better quantitative information about the extent of the infection.

To better reflect the real state in the root canal just after 15 min NaOCl irrigation, considerable attention was given to the time elapsed between irrigating and sampling in all specimens. A neutralizing agent was not applied because the sampling method employed in the present study gave little chance for the NaOCl irrigant to continue its action on the agar plates.

The average number of bacteria recovered from the irradiated teeth in the groups with small or large foramina was approximately 96% lower than the control teeth. The inability of the Er,Cr:YSGG laser when used with fixed conditions (0.5 W, with 20% air and water level, 20 s) to completely kill the bacteria can be attributable to the high degree of focus of the laser beam, the deep penetration of bacteria into the dentinal tubules because of smear layer removal prior to bacterial contamination, insensitivity of *E. faecalis* to laser irradiation because of its gram-specific cell wall structure (Moritz *et al.* 2000) and/or the resistance of starved *E. faecalis* cells to different conditions (Portenier *et al.* 2005). The laser beam delivers energy only where it is focused, thus it may not be possible to direct the beam over the entire surface of the canal wall. The bacteria may also invade the dentinal tubules, depending on morphological factors of the bacterial species. Some bacteria such as *E. faecalis* might migrate deeper into the lateral root canal or dentine tubules than others (Ragot-Roy *et al.* 1994), which may protect them from proper irradiation. Another possible factor that might prevent complete elimination of *E. faecalis* relates to the anatomy of the root canal system, which is complicated and consists of lateral canals, cul-de-sacs, fins and apical ramifications, accessory canals (Kasahara *et al.* 1990) and deltas (Diallo & Diatta 2002).

It has been shown that the Nd:YAG laser was also unable to disinfect the root canal system completely (Hardee *et al.* 1994, Fegan & Steiman 1995, Moshonov *et al.* 1995, Moritz *et al.* 2000). Two of these authors (Hardee *et al.* 1994, Moshonov *et al.* 1995) obtained CFUs with Nd:YAG laser irradiation of 98% and 99%, respectively, lower than control groups and their

results were superior to the result of Le Goff *et al.* (1999) (85%) in the evaluation of the antibacterial effect of the CO_2 laser. The superiority of the Nd:YAG laser over the CO_2 laser could be attributed to the 200 μm optical fibre directing the laser beam onto the canal wall which is considered an advantage for root canal disinfection. The 96% reduction using the Er,Cr:YSGG laser in the present study may also be attributed to the special 200 μm diameter endodontic laser fibre tip which enables better direction of the laser light into the root canals. When comparing Er,Cr:YSGG laser with the Nd:YAG laser, the latter has the disadvantage *in vivo* in that root canals must be treated with black dye to obtain maximum results because of its wavelength.

Conclusion

Er,Cr:YSGG laser reduced the viable microbial population in root canals with small and large apical foramina but did not eradicate all bacteria. Three per cent NaOCl inhibited the growth of *E. faecalis* and effectively sterilized all root canals.

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