Antibacterial Efficacy of Diode and Er:YAG Laser Irradiation in Experimentally Contaminated Primary Molar Root Canals

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Objective: *In vitro comparison of the antibacterial efficacy of Diode and Er:YAG laser irradiation with that of NaOCl irrigation in contaminated primary molar root canals.* ***Study Design:*** *96 root canals prepared from 32 extracted primary molar teeth were mechanically enlarged and the teeth were randomly divided into 4 subgroups. The roots were inoculated with an overnight culture of Enterococcus faecalis in tryptic soy broth for 24 hours. The root canals irradiated with diode and Er:YAG laser and irrigated with NaOCl (5.25%) were experimental groups and untreated canals served as positive control group. Bacterial growth was analysed by counting viable E.faecalis on tryptic soy agar plates.* ***Results:*** *The number of bacteria was significantly reduced in experimental groups in comparison with the control group. Diode laser was determined to be more effective in reducing the number of bacteria when compared to Er:YAG laser. NaOCl irrigation was found significantly most effective.* ***Conclusions:*** *Diode laser irradiation and 5.25 % NaOCl application provided a significant antibacterial effect in vitro, in contaminated primary molar root canals.*

Keywords: antibacterial effect, Er:YAG, diode, laser, primary molar, root canal
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INTRODUCTION

Despite modern advances in the prevention of dental caries and an increased understanding of the importance of maintaining the natural dentition, many primary teeth are still lost prematurely.[1] Early loss of primary teeth can cause a number of consequences including space loss for successor permanent teeth, esthetic, phonetic or functional problems.[2,3] However, some of the infected primary teeth can be kept in function until exfoliation via endodontic therapy. Pulpectomy is indicated in a primary tooth with irreversible pulpitis in which the radicular pulp exhibits clinical signs of pulp necrosis[4] or showing evidence of chronic inflammation in the radicular pulp.[1] Additionally, primary teeth with missing permanent successors represent a need for pulpectomy.[2]

The primary objective of pulp therapy is to maintain the integrity and health of the teeth and their supporting tissues.[1,4] One of the most important goals of endodontic therapy is the complete elimination of microorganisms from the root canal system. The positive correlation between bacteria and endodontic disease has been established.[5,6] The bacteriological status of the root canal before root filling is a critical factor in determining the outcome of endodontic treatment. Failure of root canal treatment is likely caused by the inability to eliminate the bacteria responsible for refractory endodontic infections.[7] *Enterococcus faecalis,* a facultatively anaerobic Gram-positive coccus is part of the human oral flora and being rarely present in primary apical periodontitis,[8] it has been implicated in persistent root canal infections and also related to failure of endodontic treatment.[9–11]

In order to achive a successful treatment outcome, mechanical instrumentation together with the use of adjunct chemical substances possessing antibacterial properties is essential.[12] Chemomechanical preparation of the root canal system using endodontic broaches and hand files,[13] together with various irrigant solutions such as: 1) saline solution; 2) sterile water; 3) chlorhexidine and sodium hypochlorite are traditionally indicated in primary root canals for the reduction of the number of bacteria.[13-15] However complete canal disinfection is difficult because of the internal complexity of root canal systems containing fins and ramifications.[12,16]

The antimicrobial effect of sodium hypochlorite (NaOCl)

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within root canals is very well documented[17-24] and it has become the most popular agent for endodontic irrigation. However no general agreement exists regarding its optimal concentration, ranging from 0.5% to 5.25%.[21] A limited effect on smear layer by NaOCl was reported and irrigation with both NaOCl and EDTA solutions has been recommended to remove the smear layer.[18,25,26]

Laser irradiation has been introduced for its potential to eliminate bacteria and thus improve endodontic treatment. Thereafter, the antimicrobial effect of different types of laser irradiation including carbon dioxide laser,[27] Nd:YAG laser.[28,29] Er,Cr:YSGG laser.[30] the argon laser,[31] Er:YAG laser[32] and Diode laser[33,34] on permanent teeth root canals have been evaluated. Lasers have been found to be relatively effective in exerting antimicrobial action,[28-30,32,33] the bacterial reduction depended on radiation energy, bacterial species,[29] time of radiation[32] and radiation frequency.[28]

Lasers have been generally used in pulpotomies of primary teeth.[35-37] To our knowledge, the antimicrobial effect of various lasers in primary teeth has not been yet evaluated. The purpose of this *in vitro* study was to compare the antibacterial efficacy of Diode and Er:YAG laser irradiation with that of NaOCl irriagation in primary molar root canals contaminated by *Enterococcus faecalis*.

MATERIALS AND METHOD

Tooth Preparation

The study sample comprised of 32 primary molars extracted for infection or excessive caries and did not have any radiographically visible physiological or pathological root resorption. Care was taken to ensure that, the teeth were not subjected to any treatment before their extraction and they were stored in sterile saline solution until the experiment, for approximately 30 days. The crowns of the teeth were reduced to the cemento-enamel junction. An access opening was prepared and the pulp was removed with a barbed broach and the root canals were enlarged using stainless steel K-files (Kerr-files; Maillefer, Ballaigues, Switzerland) up to a size of ISO 40. Saline solution was used as irrigant and the root canals were dried using paper points. The apical foramina were sealed using flowable composite resin and the teeth were embedded in acrylic resin blocks which allowed handling of the teeth during the experiment. The samples were autoclaved for 15 min at 121°C. The teeth were randomly divided into four groups and each group consisted 8 primary molars having 3 three root canals (n=24). From this stage forward, all samples were processed using strict aseptic protocols.

Contamination of root canals

A total sum of 96 root canals were inoculated with *Enterococcus faecalis* (ATCC 29212) for 24 h. A reference strain of *E. faecalis* obtained from American Type Culture Collection was used. The bacterial strain was inoculated on tryptic soy agar (TSA, Merck, Darmstadt, Germany) and incubated aerobically at 37°C for 24 h. The grown bacterial colonies were then harvested, placed in tryptic soy broth (TSB, Merck, Darmstadt, Germany), following the same incubation conditions; the turbidity of *E. faecalis* culture was adjusted to No.0.5 Mc Farland Standard. Five microliters of bacterial suspension (final concentration of about 1.5 $x10^8$ colony forming units per ml ($CFUml^{-1}$) were applied into the mechanically enlarged root canals with a sterile micropipette. The suspension was worked into the canal using a sterile endodontic file size ISO 20 for the same period for each sample (Kerr-files; Maillefer, Ballaigues, Switzerland). The opening of the canals were sealed with a temporary filling material (Coltosol® Coltene Whaledent). All samples were stored at 37°C for 24 h. under aerobic conditions.

Irradiation and disinfection procedures

Two sources of laser radiation were used for the disinfection of root canals. The first group was irradiated using an 810 nm diode laser device (HOYA ConBio diyotent II). The laser radiation was applied in continuous mode with a 300 mW energy. The laser beam was transferred to the handpiece via a flexible glass fiber; in the handpiece it is coupled to the actual application tip with an outer diameter of 300μm. No irrigation was done before irradiation, the optic fiber was inserted up to the working length and pushed up and down along the root canal in three consecutive cycles of 15 s. The second group was irradiated using an Er:YAG device (HOYA ConBio VersaWave) emitting pulsed infrared radiation at a wavelength of 2.94μm. The laser radiation was applied at a defined pulse energy of 50 mJ and with a constant repetition rate of 15 Hz. The laser radiation was delivered into the root canals using an endodontic handpiece and a flexible quartz glass fiber with an outer diameter of 200μm. The root canals were irrigated with 1 ml. sterile saline solution before Er:YAG laser application and afterwards, the fiber was inserted up to the working length and moved in three consecutive cycles from apical to coronal for 15 s. In the third group, disinfection was carried out by irrigating with 1 ml. 5.25% NaOCl solution, three serial rinses were done with a 5 min contact period between bacteria and irrigant. The fourth group was left untreated for positive controls. Additionally, one tooth with three root canals was inoculated with sterile TSB and served as a negative control.

After the disinfection procedures, the root canals in all groups were rinsed with 1 ml. sterile saline solution. The saline solution was collected from canals with sterile paper points for a standard 15 s. contact for sample collection. The paper points were transferred to Eppendorf vials containing 1 ml of VMG II transport fluid.[38] All collected samples were vortexed for 10 seconds and 10-fold dilutions were prepared. Aliquots of 0.1ml suspensions were inoculated on TSA plates and incubated at 37°C for 24h. Colony forming units per ml ($CFU\ ml^{-1}$) were enumerated for per root canal sample.

Statistical Analysis

To obtain a near normal distribution, tha data for CFU has been subjected to logarithmic transformation. The statistical analysis was performed using the NCSS 2007 software program. Besides the descriptive statistical methods (geometric mean and median), one-way ANOVA was used for the comparison of the groups and Tukey multiple comparison test was used for the subgroup comparisons.

RESULTS

The samples in the negative control group exhibited no formation of bacterial colonies. The geometric mean and median of remaining bacteria in the positive control and experimental groups and the comparison of groups according to one-way ANOVA are presented in Table 1. The untreated positive control group revealed the highest number of bacteria ($406*10^5$ CFU ml^{-1}) and NaOCl exhibited the highest antimicrobial effect in all three experimental groups (0 CFU ml^{-1}). The comparison between the groups showed a statistically significant difference ($p<0,0001$). Table 2. represents the comparison of groups according to Tukey multiple comparison test. The statistical analysis showed a significant difference between the two laser groups and Diode laser ($0,4*10^5$ CFU ml^{-1}) was found more effective than the Er:YAG laser ($18*10^5$ CFU ml^{-1}) ($p<0,05$) and also NaOCl was found significantly more effective than the Er:YAG laser ($p<0,001$). Although the number of bacteria found in the Diode laser group was higher than the NaOCl group, the difference was not significant.

Table 1. The geometric mean number and median of remaining bacteria in the control and experimental groups and the comparison of groups according to one way ANOVA.

	Geometric Mean	Median
Control	406*105	565*105
DIODE Laser	0,4*105	0,25*105
Er:YAG Laser	18*105	59*105
NaOCl	0	0
F	54,79	
p	0,0001	

Table 2. The comparison of subgroups according to Tukey's multiple comparison test

Tukey's Multiple Comparison Test	P value
Control / DIODE Laser	**P < 0.001**
Control / Er:YAG Laser	**P < 0.01**
Control / NaOCl	**P < 0.001**
DIODE Laser / Er:YAG Laser	**P < 0.05**
DIODE Laser / NaOCl	**P > 0.05**
Er:YAG Laser / NaOCl	**P < 0.001**

DISCUSSION

In the present 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, since it has been frequently recovered from the root canals of teeth associated with post-treatment diseases[8-11] and persistent apical periodontitis.[39] It was also reported to be resistant to intracanal medicaments such as calcium hydroxide.[40,41] In a PCR-based in vivo study Cogulu et al.[42] evaluated the presence of various pathogens in the root canals of primary and permanent teeth and determined that *E. faecalis* and *T.denticola* were highly associated with periapical radiolucency and previous pain in both primary and permanent teeth.

The antimicrobial efficacy of various lasers against *E. faecalis* in permanent teeth have been evaluated and documented in the English dental literature. Eldeniz *et al*[30] showed that Er,Cr: YSGG laser reduced the viable microbial population in root canals, but could not eradicate all bacteria, nonetheless 3% NaOCl inhibited the growth of *E. faecalis* and provided complete elimination of the bacteria in all root canals. 5.25% NaOCl showed a similar antibacterial effect in the present study.

In an *in vivo* study Moritz *et al*[33] determined that irradiation with a 810 nm Diode laser in two subesequent sessions resulted in nearly complete elimination of bacteria and suggested that the diode laser can be considered equal to the Nd:YAG laser in endodontic treatment. The Diode laser was found more effective than the Er:YAG laser, and nearly as effective as NaOCl in this study. In some of the samples Diode laser revealed a complete elimination of *E.Faecalis*.

Schoop *et al* [43] determined that Er:YAG laser was also a suitable tool for the elimination of bacteria in root canals under in vitro conditions. Irradiation with different energy settings (0.5 W, 1 W, 1.3 W) resulted with a distinct reduction in bacterial counts of different species except *Enterococcus faecalis*. It was suggested that the complete eradication of *E. faecalis* would require power settings that bear the risk of severe thermal side effects and ultimately damage to the surrounding periodontal tissue. However, it was also mentioned that, the special characteristics of Er:YAG laser radiation lead to a steep temperature gradient, thus causing no thermal side-effects in the surrounding tissues.[32] It is recommended to apply the hard tissue preparation lasers like Er:YAG and Er,Cr:YSGG with water or air cooling since they lack the large range of acceptable power settings of the shorter wavelengths when applied in root canal otherwise.[44] In the present study irradiation with Er:YAG laser was applied at a defined pulse energy of 50 mJ and with a constant repetition rate of 15 Hz, which is equivalent to 0.75 W. The root canals in the Er:YAG laser group was irrigated with 1 ml. sterile saline solution before and after laser irradiation, according to the manufacturer's instructions, in order to prevent temperature rise and a significant bacterial reduction was achieved for *E.faecalis* with these settings, which is a similar finding with those of Schoop *et al*.[43]

It was also determined that the duration of radiation is a determining factor in the antimicrobial effectiveness of Er:YAG laser. Irradiation for 60 sn. with Er:YAG laser was found nearly as effective as rinsing with 1.25% NaOCl solution for 2 min. It was emphasized that laser treatment

can lead to a similar removal of bacteria when irradiating the root canals for at least 60 s.[32] The irradiation time used in this study was a total of 45 s. consisting of three consecutive cycles of 15 s. However the NaOCl solution was higher in concentration (5.25 %), thus a significantly higher reduction in the number of bacteria was obtained. From another point of view, a highly concentrated NaOCl solution could be irritating while treating a pediatric patient; a prolonged irradiation with Er:YAG laser might be an alternative measure for the endodontic treatments of children.

There are only a few studies comparing different laser systems with regard to their antimicrobial properties. Moritz *et al* [45] have compared the Nd:YAG, the Er:YAG, and the Ho:YAG lasers under *in vitro* conditions. The Er:YAG laser was reported to be most effective in bacterial eradication. Thereafter, Schoop *et al*.[44] compared the bactericidal effect of Nd:YAG, the diode, the Er:YAG, and the Er, Cr: YSGG laser in the deep layers of dentin. The elimination of gram-negative bacteria like *E.coli* was reported to be easier to achieve than that of gram- positive strains like *E. faecalis*. The massive cell-wall-structure of this species was considered to be responsible for its resistance. Furthermore, the four tested laser systems were shown to be effective in disinfecting the dentin samples and again best results were obtained with Er:YAG laser. The present study was designed with the aim of evaluating laser irradiations' effectiveness in root canal treatments of primary teeth, but also two different laser systems with two different wavelengths were compared. The results exhibited that the diode laser was siginificantly more effective than the Er:YAG laser in reducing the number of bacteria. However, both lasers were insufficient in eradicating all bacteria. The contradiction with the previous studies might result from the difference in the study design, also the complexity of the primary molar root canal system might have presented a difficulty for the lasers in reaching bacteria invading dentin walls and tubules.

Under *in vivo* conditions, the microflora of an infected root canal consists of multiple types of microorganisms which 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. Although 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 done regarding the influence of different disinfection protocols. Although care was taken to standardize the conditions of assessment of the three disinfection methods evaluated in this study, due to the differece in the mechansims of action for each group, some variations with regard to the technique are always expected.

The antimicrobial effectiveness of NaOCl in root canals is reported to be a function of concentration and contact time.[21,23,24] Nevertheless, Siqueira *et al* [23] suggested that, low concentrations of NaOCl may significantly reduce the endodontic infection, but might not consistently dissolve all remnants in a reasonable time and the efficacy of weak solutions may decrease rapidly. *E.faecalis* was found to be more resistant to NaOCl than *Actinomyces naeslundii* and *Candida albicans*.[24] In a previous study Gomes *et al* [21] showed that 5.25% was the most efficient concentration of NaOCl in the elimination of *E. Faecalis* in less than 30 seconds. A high concentration NaOCl (5.25%) solution was selected for the disinfection of primary root canals and a contact time of 5 min. was administered in this study in order to achieve comparable results with lasers evaluated.

In the previous studies, effectiveness of various instrumentation and cleaning methods in primary teeth have been evaluated. Ultrasonication was proposed as a useful adjunct for endodontic cleaning of primary teeth and it was suggested that ultrasound is more effective than conventional hand filing in the debridement of primary root canals which are hardly accesible to mechanical cleaning.[46] In the following years, the use of rotary instrumentation for primary teeth pulpectomies have gained attention and was accepted as an effective way to debride the uneven walls of primary root canals.[47,48]

The use of lasers in the endodontic therapies of primary teeth has not been yet evaluated. The methodologies used in the *in vitro* studies which investigate the antimicrobial effectiveness of lasers include only anterior permanent teeth with single root canals. Primary molars with at least three root canals were included in the present study, since root canal treatment is often indicated in these teeth representing irreversible pulpitis, chronic inflammation, or with missing successors. Additionally, the study was designed to highlight the viability of lasers in the endodontic therapies of primary molars and determine whether they can be suggested as an supplements to irrigation with NaOCl.

CONCLUSIONS

Under the experimental conditions and within the limitations of this study the following conclusions can be made:

1. Diode laser irradiation and 5.25 % NaOCl application provided a significant antibacterial effect in experimentally contaminated primary root canal.
2. Diode laser was found to be more effective in reducing the number of bacteria in comparison with Er:YAG laser. However, both lasers were insufficient in eradicating all bacteria.
3. The results of the study should be confirmed with clinical investigations in order to suggest laser irradiations for the disinfection of root canals of primary teeth.

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