Original Research

Diode Laser and Calcium Hydroxide for Elimination of Enterococcus Faecalis in Root Canal

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Abstract

Introduction: The ultimate goal of endodontic treatment is to eliminate the bacterial infection in the root canal system. While mechanical debridement combined with chemical irrigation removes the bulk of microorganisms, residual bacteria are readily detectable in approximately one-half of teeth just prior to obturation. Laser light can be used to destroy bacteria. This in vitro study was performed to evaluate the effect of diode laser and calcium hydroxide on mono-infected dental canals.**Methods:** Fifty five single-rooted human premolars were prepared and contaminated with Enterococcus faecalis. After three weeks of incubation, the samples were divided into three experimental groups (n = 15) and two control groups (n = 5). In the first and second groups, the teeth were rinsed for 5 min with either sterile saline or 5.25% NaOCl and irradiated with a 810-nm diode laser at 1.5 W output for 5 × 4s. In the third group, the teeth were rinsed with 5.25% NaOCl and then $Ca(OH)_2$ paste was inserted in the canals for 1 week. Intracanal bacterial sampling was done and the samples were plated to determine the CFU count. **Results:** 5.25% NaOCl plus laser was as effective as calcium hydroxide and significantly more effective than sterile saline ($P>0.05$) in elimination of E. faecalis. Complete elimination of E. faecalis was seen only for the one week calcium hydroxide treatment. **Conclusion:** Combination therapy with NaOCl irrigation and diode laser irradiation can be recommended as an effective treatment option for elimination of E. faecalis from the root canal system.

Key words: Antibacterial properties, Calcium hydroxide, Diode laser, Enterococcus faecalis.

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Introduction

The purpose of root canal therapy is its disinfection and sealing to prevent recurrent infections. Presence or absence of microorganisms in root canals during obturation is the main factor, influencing the outcome of endodontic treatment (1,2). In other words, according to the role of micro-organisms in apical periodontitis, endodontic treatment should focus on removal of microbial colonization from root canal system (through antiseptic procedures) and preventing the entry of new micro-organisms to the root canals (by aseptic techniques) (3). The success of endodontic treatment depends on whether the clinician succeed in achieving these goals or not (4,5).

After pulpal infection, bacteria can penetrate into the dentinal tubules as well as periapical tissue. Chemo-mechanical methods of canal preparation are not able to eradicate bacteria from root canals and dentinal tubules. Approximately 40-60% of microorganisms can survive through chemo-mechanical preparation, which even includes antimicrobial irrigation (6-8). This might be due to their inaccessible location in isthmuses, additional canals and apical region (6).

On the other hand, common irrigators used during endodontic treatment, such as sodium hypochlorite, affect via direct contact to the bacteria. As result,the bacteria which penetrate to the deep layers of dentin can be immune, since irrigator's depth of penetration is limited (9,10).

Studies have demonstrated that predictable disinfection of root canal system can only be achieved when inter appointment antimicrobial medicament is placed in root canals (11-13).

Researchers tested different laser systems to achieve a more complete disinfection of root canal system and adjacent dentinal tubules and yet there is still controversy about the most powerful laser system in regards to provide a sterilized root canal. High bactericidal effect of Nd-YAG laser and diode laser has been reported in different studies (14).Consequently, due to the bactericidal effect of diode laser and its low cost compared to other endodontic common laser systems, it can be used along with the mechanical debridement procedures.

Laser can be transported into canals using fiber optics such as Nd-YAG, argon and diode or with hollow tubes such as CO_2 laser and Er-YAG. These different delivery methods can change the potency of similar lasers in canals' sterilization.

Use of laser in endodontic treatment has greatly enhanced success rate of treatment and the reason may be due to the laser's ability to penetrate to deeper dentinal layers (15,16).

Therefore, the present experimental study was designed to compare the effect of calcium hydroxide with laser diode plus 5.25% sodium hypochlorite and laser diode plus saline in removal of Enterococcus faecalis from root canal system.

Materials and Methods

Fifty five extracted human single-rooted mandibular premolars were used in current study (three experimental groups of 15 teeth and two control groups of 5 teeth). Exclusion criteria included premolars with more than one root canal or root canal curvature of more than 25, the root surface's decay and the teeth with calcified canals. After extraction of teeth, their debridement was done by handle instruments and they were kept in normal saline until the experiment day. All crowns were cut from CEJ to achieve a similar root length for all samples (15 mm). In order to determine working length, #15 K-file (Dentsply Maillefer, Ballaigues, Switzerland) was placed in root canals. When the file tip was seen at the apex of the tooth, the working length was recorded by subtracting one millimeter. Afterward, instrumentation was continued with #20 and 25 K-files to remove the pulp tissue and homogenizing the samples. Irrigation was done with 5^{cc} of 5.25% sodium hypochlorite after each file application. Then, all samples were autoclaved at 134 °C for 15 min. At this stage, five teeth were randomly selected and were kept as negative control.

Bacterial inoculation

Standard suspension of Enterococcus faecalis (ATCC 29212) containing 1.5×10^8 bacteria per ml was prepared and samples were placed into the suspension for 21 days so the bacteria can penetrate fully into the dentinal tubules and form biofilm (17). To meet the needs of growing bacteria, culture medium was replaced every 3 days. After this stage, 5 teeth were randomly kept as positive control.

Laser irradiation

The remaining 45 teeth were randomly divided into three groups. In all samples instrumentation was done with ProTaper rotary files system (Dentsply, Tulsa Dental, Tulsa, USA), in the way that Sx was the first file to widen the coronal third of the canal, followed by files F2 and F3 to reach the working length.

Irrigation and laser irradiation of canals was done according to the divided groups as follows:

Group I: The irrigation during preparing canals and in the final stage was done with 5cc sterile saline solution. The diode laser (GmbH ARC Laser, Germany) with a wave length of 810 nm and a power of 1.5W, was then beamed into the canal, in the way that the optical fiber reached up to 1 mm shorter than working length and was rotated with the speed of 2 mm per second, for 5 seconds. This was repeated for four times with a10-second interval between.

Group II: The irrigation during the preparation was done with 5cc of 5.25% NaOCl followed by a final irrigation with saline. At the end, laser was beamed into the canals similar to the first group.

Microbial sampling was performed using size 30 sterile paper points (Aria Dent, Tehran, Iran) placed inside the root canal for 1 min and then transferred to test microtubes containing 1 ml of sterile brain heart infusion broth culture. In order to determine the bacteria within the dentinal tubules, after sampling with paper point, the canals were prepared with sterile Gates Glidden drills # 5 and the samples were taken again (17). The microtubes were vortexed for 30 seconds. 10 ml was removed for counting the number of surviving bacteria on the surface of solid medium and incubated. Cultural plates were evaluated after 24h incubation. Bacterial growth was measured by the CFU/mL counts of E. faecalis.

In groups I and II, bacterial sampling was done immediately after irradiation.

Group III: The irrigation during the preparation was done with 5cc of 5.25% NaOCl. Calcium hydroxide mixed with sterile saline in equal parts and paste

inserted into dried canals with the help of lentulo spirals (Dentsply Maillefer, Ballaigues, Switzerland). Cavities were sealed with Cavit (ESPE Dental AG, Seefeld, Germany).A week later, the root canals were irrigated with 5 mL of sterile saline solution and agitated with a size 30 K-file up to the working length in order to remove the intracanal medication. The post-medication samples were collected as previously described. The number of CFU/mL was calculated.

In the entire process, sterile instruments were used in order to prevent unwanted contamination of canals. One way ANOVA or Kruskal-Wallis test was used to analyze the data.

Results

No colonies were found on agar plates in the negative control group to ensure that no contamination of the samples during the experimental period had happened. Extensive growth of bacteria in all samples in the positive control group (CFU = 150000) was observed.

Means and standard deviations of CFU in all groups are shown in Table 1.In calcium hydroxide group, no bacterial colonies were observed in any of the samples. The group treated with saline and laser, had zero as minimum number of bacterial colonies and 12000 CFU/ml as maximum, which was a fairly large scatter. In the group disinfected with sodium hypochlorite and laser, the minimum number of bacterial colonies was zero and the maximum was 3000 which was still scattered but it was less than saline –laser group. In general, the number of colonies was significantly different between the tree groups and Mann-Whitney test with Bonferroni correction revealed, difference of colony counts between calcium hydroxide group and hypochlorite-laser group was not statistically significant. But number of colonies in group treated with saline-laser was significantly higher than both other groups.

Discussion

The success of endodontic treatment depends basically on elimination of the bacteria in root canal system and preventing their re-entry. The remained bacteria after treatment can affect long-term outcome of root canal therapy (3). Despite the proved efficacy of various lasers against microorganisms, studies in regards to laser's antimicrobial ability in root canal system, are controversial. The most of previous studies have shown a fairly high capability of disinfecting dentinal tubules, However complete elimination of endodontic pathogens, especially bacterial species that grows biofilms, has not been achieved (18,19) and further application of these methods with other common antiseptic solutions have been suggested in some studies (20-22). Diode laser has high permeability and low interference with dentin, which allows the laser to be effective on microorganisms that have penetrated into the dentinal tubules(23).

Bactericidal capability of the diode lasers on different bacterial species, including Enterococcus faecalis, has been demonstrated by several authors (24-27).

Bacteria such as Enterococcus faecalis (which is a predominant bacterial species in resistant endodontic infections), are very resistant to endodontic common irrigation solutions (28) and they were isolated from canals with persistent infections and endodontic failures, in several studies (7,29,30).

Table1. Mean and standard deviation of CFU/ml and degree of disinfection in the experimental groups, relative to the positive controls

Group	Number of samples	Average number of colonies	Standard deviation	Minimum number of colonies	Maximum number of colonies	Test results	Disinfection capability (%)
Calcium hydroxide	15	.0000	.00000	.00	.00	X^2=26.07	100
Saline-laser	15	2012.1429	4252.3553	.00	12000.0	P<0.001	98.65
Hypochlorite-laser	15	206.6667	773.18143	.00	3000.00		99.86

In an *ex vivo* study, E. faecalis survived for 12 months in root canal and in addition it was showed that E. faecalis has the ability to convert to alive but non-cultivable situation, (VBNC; viable but not cultivable) which is a strategy to survive through stressful conditions (31,32). In VBNC situation, changes in cell wall occur which may provide protection against in adequate environmental conditions (33) in addition to maintain the ability to adhere to cultured human cells (34). These conditions include the presence of sodium hypochlorite, salts, bile salts, acids, alkaline, stress, and absence of glucose and increase of temperature (35).Different studies showed that enterococci could even be isolated from properly treated root canals, which may be due to low sensitivity to antimicrobial agents and the ability to with stand high PH (including calcium hydroxide paste with PH> 11.5) (36,37).

Since some studies has been shown that the use of laser in canals, irrigated with antiseptic agents or with saline alone are different with each other (20), in this study to further simulate the clinical condition, both types of irrigations were used in combination with laser.

In the present study, according tothe significantly lower mean number of bacteria in hypochlorite–laser group compared to saline- laser group (206 vs.2012),it can be concluded the presence of hypochlorite had an important role in increasing the efficiency of diode laser to destroy bacteria. In addition, regards to the concept that efficiency of diode laser would be increased when it's accompanied with other irrigations, de Souza EB et al. (38) in their study stated that the radiation of high power diode laser (wavelength 830 nm and power 3 W) followed by irrigation with 0.5% hypochlorite and17% EDTA-T, will lead to an increased disinfection in deeper layers of root dentin. Results of Kreisler etal.(26) emphasized on the combined use of diode laser and irrigation solutions like NaOCl and H2O2 in endodontic treatments. Moreover, the study of Mehrvarzfar et al. showed that diode laser with use of Biopure MTAD an eradicate Enterococcus faecalis totally from the root canals (20).

In the group treated with saline plus laser, the mean bacterial colony was 2012 and according to the colonies of the positive control group (150,000), a 98.6% decrease of bacteria was seen in this group. In this regard, some other studies have suggested that the laser diode is notable to completely remove Enterococcus faecalis (18,24,25). Moritz et al. have shown that complete elimination of bacteria only happens when high power laser is used and this may lead to higher temperature on root surface (27). In Mehrvarzfar study, diode laser with a power of 2 watt was used after irrigation with saline, and it was only 80 percent effective in disinfection of canals (20), while some studies have said that the antibacterial ability of diode laser on dentin blocks with a thickness of 100 micrometers is more than 95 percent (24,25). This difference may be due to the use of dentinal pieces and immediate sampling after laser irradiation, while in Mehrvarzfar experiment, sampling was performed 24 h after laser irradiation and this may provide the time required for regrowth of survived bacteria (20). In the present study, sampling was performed immediately after laser irradiation, and this could cause results to differ with the results of Mehrvarzfar study.

Since the analysis revealed that the antibacterial effect of diode laser with parameters used in this study (810 nm, 1.5W) was enhanced in combination with 5.25% sodium hypochlorite, and this group did not have a significant difference with the group treated with calcium hydroxide, for patient's comfort, clinician can use this strategy in order to disinfect root canal systems as an alternative for the calcium hydroxide medicament in persistent endodontic infections. However, due to the possible harmful effects of lasers on periapical and periodontal tissues, particularly when used in high intensity, further clinical studies should be conducted to understand more fully damage. In addition, in order to understand more about the parameters affecting laser's properties, it is necessary that further studies with other parameters of the laser radiation be conducted.

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References

1. Fabricius L, Dahlen G, sundqvist G, Happonen RP, Möller AJ. Influence of residual bacteria on periapical tissue chemomechanical treatment and root filling of experimentally infected monkey teeth. Eur J Oral Sci 2006;114:278-85.
2. Engström B, Lundberg M. The correlation between positive culture and the prognosis of root canal therapy after pulpectomy. Odontol Revy 1965;16:193-203.
3. Ingle JI, Bakland LK, Baumgartner JC. Ingle's Endodontics. Hamilton: B.C. Decker Inc, 2008.
4. Sjogren U, Hagglund B, Sudqvist G, Wing K. Factors affecting the long-term results of endodontic treatment. J Endod 1990;16:498-504.

5. Bystrom A, Happonen RP, Sjogren U, Sundqvist G. Healing of periapical lesions of pulpless teeth after endodontic treatment with controlled asepsis. Endod Dent Traumatol 1987;3:58-63.
6. SjogrenU, Figdor D, Persson S, Sundqvist G. Influence of infection at the time of root filling on the outcome of endodontic treatment of teeth with apical periodontitis. Int Endod J 1997;30:297-306.
7. Bystrom A, Sundqvist G. The antibacterial action of sodium hypochlorite and EDTA in 60 cases of endodontic therapy. Int Endod J 1985;18:35-40.
8. Shuping GB, Orstavik D, Sigurdsson A, Trope M. Reduction of intracanal bacteria using nickel-titanium rotary instrumentation and various medications. J Endod 2000;26:751-5.
9. Berutti E, Marini R, Angeretti A. Penetration ability of different irrigants into dentinal tubules. J Endod 1997;23:725-7.
10. Kouchi Y, Ninomiya J, Yasuda H, Fukui K, Moriyama T, Okamoto H. Location of Streptococcus mutans in the dentinal tubules of open infected root canals. J Dent Res 1980;59:2038-46.
11. Bystrom A, Claesson R, Sundqvist G. The antimicrobial effect of camphorated paramonochlorophenol, camphorated phenol and calcium hydroxide in the treatment of infected root canals. Endod Dent Traumatol 1985;5:170-5.
12. Sjogren U, Figdor D, Spangberg G. The antimicrobial effect of calcium hydroxide as a short-term intracanal dressing. Int Endod J 1991;24:119-25.
13. McGurkin-Smith R, Trope M, Caplan D, Sigurdsson A. Reduction of intracanal bacteria using GT rotary instrumentation , 5.25% NaOCL, EDTA, and $Ca(OH)_2$. J Endod 2005;31:359-63.
14. Schoop U, Goharkhay K, Klimscha J, Zagler M, Wernisch J. The use of the erbium, chromium: yttrium scandium-gallium-garnet laser in endodontic treatment; The results of an in vitro study. JAm Dent Assoc 2007;138:949-55.
15. Vaarkamp J, ten Bosch JJ, Verdonschot EH. Propagation of light through human dental enamel and dentine.Caries Res 1995;29: 8-13.
16. Odor TM, Chandler NP, Watson TF, Ford TR, McDonald F. Laser light transmission in teeth: a study of the patterns in different species. Int Endod J 1999;32:296-302.
17. Javidi M, Zarei M, Afkhami F. Antibacterial Effect of Calcium Hydroxide on Intraluminal and Intratubular Enterococcus Faecalis. Iran Endod J 2011;6:103–6.
18. Schoop U, Kluger W, Moritz A, Nedjelik N, Georgopoulos A, Sperr W. Bactericidal effect of different laser systems in the deep layers of dentin. Lasers Surg Med 2004;35:111-6.
19. Bergmans L, Moisiadis P, Teughels W, Van Meerbeek B, Quirynen M, Lambrechts P. Bactericidal effect of Nd:YAG laser irradiation on some endodontic pathogens ex vivo. Int Endod J 2006;39:547-57.
20. Mehrvarzfar P, Saghiri MA, Asatourian A, et al. Additive effect of a diode laser on the antibacterial activity of 2.5% NaOCl, 2% CHX and MTAD against Enterococcus faecalis contaminating root canals: an in vitro study. J Oral Sci 2011;53:355-60.
21. Sahar-Helft S, Stabholtz A, Moshonov J, Gutkin V, Redenski I, Steinberg D. Effect of Er:YAG laser-activated irrigation solution on Enterococcus Faecalis biofilm in an ex-vivo root canal model. Photomed Laser Surg 2013;31:334-41.
22. Shabahang S, Torabinejad M. Effect of MTAD on Enterococcus faecalis-contaminated root canals of extracted human teeth. J Endod 2003;29:576-9.
23. Klim JD, Fox DB, Coluzzi DJ, Neckel CP, Swick MD. The diode laser in dentistry. Rev Wavelengths 2000;8:13-6.
24. Gutknecht N, Franzen R, Schippers M, Lampert F. Bactericidal effect of a 980-nm diode laser in the root canal wall dentin of bovine teeth. J Clin Laser Med Surg 2004;22:9-13.

25. Gutknecht N, van Gogswaardt D, Conrads G, Apel C, Schubert C, Lampert F. Diode laser radiation and its bactericidal effect in root canal wall dentin. J Clin Laser Med Surg 2000;18:57-60.
26. Kreisler M, Kohnen W, Beck M, et al. Efficacy of NaOCl/H2O2 irrigation and GaAlAs laser in decontamination of root canals in vitro. Lasers Surg Med 2003;32:189-96.
27. Moritz A, Gutknecht N, Goharkhay K, Schoop U, Wernisch J, Speer W. In vitro irradiation of infected root canals with diode laser: results of microbiologic, infrared spectrometric, and stain penetration examinations. Quintessence Int 1997;28:205-9.
28. Estrela C, Estrela CRA, Decurcio DA, Hollanda ACB, Silva JA. Antimicrobial efficacy of ozonated water, gaseous ozone, sodium hipochlorite and chlorhexidine in infected human root canals.IntEndod J 2007;40:85–93.
29. sundqvist G, Figdor D, person S, Sjogren U. Microbiologic analysis of teeth with failed endodontictreatment and the outcome of conservative re-treatment. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1998;85:86-93.
30. Siqueira JF JR, Rocas IN. Polymerase chain reaction-based analysis of microorganisms associated with failed endodontic treatment. Oral Surg Oral Med Oral Pathol Oral Radiol Oral Endod 2004;97:85-94.
31. Lieo MM, Pierobon S, Tafi MC, Signoretto C, Canepari P. mRNA detection by reverse transcription –PCR for monitoring viability over time in an Enterococcus faecalis viable but nonculturable population maintained in a laboratory microcosm. Appl Environ Microbiol 2000;66: 4564-7.
32. Lieo MM, Bonato B, Tafi MC, Signoretto C, Boaretti M, Canepari P. Resuscitation rate in different enterococcal species in the viable but nonculturable state. J Appl Microbiol 2001;91:1095-102.
33. Signoretto C, Lleo MM, Tafi MC, Canepari P. Cell wall chemical composition of Enterococcus faecalis in the viable but nonculturable state. Appl Environ Microbiol 2000;66:1953-9.
34. Pruzzo C, Tarsi R, Lleo MM, et al. In vitro adhesion to human cells by viable but nonculturable Enterococcus faecalis. Curr Microbiol 2002;45:105-10.
35. Giard JC, Laplace JM, Rince A, Pichereau V, Benachour A, Leboeuf C, Flahaut S, Auffray Y, HartkeA.The stress proteome of Enterococcus faecalis. Electrophoresis 2001;22:2947-54.
36. Portenier I, Waltimo TMT, Haapasalo M. Enterococcus faecalis – the root canal survivor and ‘star’ in post-treatment disease. Endod Topics 2003;6:135-59.
37. Appelbe OK, Sedgley CM. Effects of prolonged exposure to alkaline pH on Enterococcus faecalis survival and specific gene transcripts. Oral MicrobiolImmunol 2007; 22:169-74.
38. De Souza EB, Cai S, Simionato MR, Lage-Marques JL. High-power diode laser in the disinfection in depth of the root canal dentin.Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2008;106:e68-72.

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