Comparative evaluation of the efficacy of laser and plasma microjet on root canals infected with enterococcus faecalis - An in vitro-study

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Abstract

Introduction: Aim of this in vitro study was to compare the anti-microbial efficacy of diode laser and plasma microjet with sodium hypochlorite in root canals infected with E. faecalis.

Materials and Method: Teeth were sterilized after decoronation and preparation of the root canals. A liquid culture suspension of 0.5 McFarland standard BHI broth was prepared to obtain 1.5×10^8 colony forming units/ml from a subculture of E.faecalis. Sterilized teeth were incubated with the bacterial suspension for a week under aerobic and static conditions at 37°C. Based on their treatment, the samples were divided into 2.5% sodium hypochlorite (control group, n=15), diode laser (n=15) and plasma microjet (n=15). Sterile paper points were introduced to working length, allowed to saturate, and were transferred to tubes containing 1 ml of freshly prepared BHI broth and vortexed for 20 seconds. After 10 fold serial dilutions, aliquots of 0.1 ml were plated onto blood agar plates and incubated. The colony forming units grown were counted and recorded.

Result: There was a statistically significant difference observed among the 3 groups using ANOVA test. Further Tukey's post hoc analysis showed significant difference between groups A & C, and groups B & C. No difference was found between groups A & B.

Conclusion: Diode laser and sodium hypochlorite proved to be equally efficacious in their anti-microbial properties against E. faecalis. Plasma microjet exhibited least antimicrobial activity as compared with diode laser and sodium hypochlorite.

Keywords: Sodium hypochlorite, Diode laser, Plasma, Anti-microbial efficacy, E. faecalis

Introduction

Successful root canal therapy relies on the combination of proper instrumentation, irrigation, and obturation of the root canal. Of these three essential steps of root canal therapy, irrigation of the root canal is the most important determinant in the healing of the periapical tissues. The success of a root canal treatment depends on thorough chemomechanical debridement of pulpal tissue, dentin debris, and infective microorganisms. Bacterial infection has long been recognized as the primary etiologic factor in the development of pulpal and periapical lesions.(1)

The purpose of root canal treatment is to eliminate entirely the infection of the root canal system and prevent reinfection. Many studies have shown that persistent endodontic infections are frequently caused by Enterococcus faecalis.(2) E. faecalis is a facultative anaerobic Gram positive coccus, which is present in oral flora and is identified in persistent root canal infections and is also related to the failure of endodontic treatment.(3) They form intra and extra radicular biofilms, which are difficult to remove, hence causing persistent infections.(4)

Numerous irrigants have been recommended for use in the treatment of root canal infections. Sodium hypochlorite (NaOCl) has been widely used as an irrigant since its introduction in endodontics by Walker in 1936. In addition to bleaching, deodorizing and tissue-dissolving properties, NaOCl has been demonstrated to be an effective disinfectant agent.(5) Although NaOCl, as endodontic irrigant, has a superior antibacterial efficacy and is accepted as 'gold standard', its cytotoxic properties force the researchers to innovate the current irrigation regimen.(6)

The application of plasma in sterilization of a root canal of a tooth has recently attracted great attention. When the electrons are stripped from atoms and molecules, they enter into a high energetic state called plasma, which is the fourth state of matter.(7) Plasma consists of positively and negatively charged ions and negatively charged electrons as well as radicals, neutral and excited atoms and molecules.(8) There are recent studies investigating its efficacy against endodontic micro-organisms in vitro(9,10) or for root canal disinfection ex vivo.(11) The biggest advantage of plasma is its capability to reach deep into the infected sites in the complex root canal system.

Lasers have become latest choice to eradicate microorganisms in the root canal, especially in the lateral dentinal tubuli. It has been proved in numerous studies that an emission of laser light directly in the root canal does have bactericidal effect.(12) The antibacterial effect of a laser beam is based on thermal properties of laser tissue interaction.(13) Diode laser has been proved to be resource worth testing.

The primary aim of this study is to compare the antimicrobial efficacy of diode laser and plasma microjet with 2.5% NaOCl on root canals infected with Enterococcus faecalis.

Materials and Method

Forty five permanent human anterior teeth with single root extracted for periodontal reasons, were used for the study. The teeth were decoronated at the cemento-enamel junction using high-speed diamond disk to obtain roots 14 mm in length. Conventional access cavity preparations were done, and all the canals were cleaned and shaped by rotary nickel-titanium ProTaper instruments (Dentsply Maillefer, Ballaigues, Switzerland) until F2. The canals were irrigated with 1 mL 2.5% sodium hypochlorite (NaOCl) after each instrument size. A lubricant (EDTA, RC help) was used throughout the cleaning and shaping of the root canal. All root apices were then sealed with nail varnish. Finally, the root canals were autoclaved at 121˚C for 15 minutes at 15 lbs pressure. After sterilization, roots were incubated in brain–heart infusion (BHI) broth for 48 h at 37°C to ensure that there is no bacterial contamination. **[Fig. 1]**

A liquid culture suspension of 0.5 McFarland standard BHI broth was prepared to obtain 1.5 X 10^8 colony forming units per mL from a subculture of E. faecalis. The sterilized tooth was placed in an Eppendorf tube, 1 ml of the bacterial suspension was added into this tube and it was incubated for a week under aerobic and static conditions at 37°C. **[Fig. 2]** The medium was changed every 2 days to avoid saturation and confirmed the growth of E. faecalis, and the cultures were checked for purity by Gram stain and colony morphology on BHI agar with 10% sheep blood. After the incubation period, roots were assigned randomly to A, B or C groups.

Fig. 1: Roots incubated in sterile BHI broth

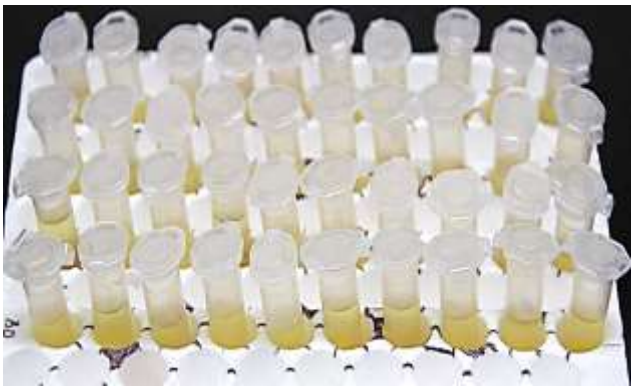


Fig. 2: Roots placed in E. faecalis suspension in eppendorf tubes

The treatment protocol for each group was as follows:

- **Group A (n= 15):** Control group; 2.5% NaOCl was used.
- **Group B (n=15):** In this group, the specimens were treated with diode laser.
- **Group C (n=15):** In this group, the specimens were treated with plasma microjet.

In group A, the root canals were irrigated with 5 mL 2.5% NaOCl for 60 s using 5-mL syringe and 26-G needle, which was placed 2 mm short of the WL. In group B, the root canals were irradiated with a diode laser (iLase, Biolase, California, USA) with an endodontic tip (ezTipEndo, 20mm/200µm). Specimens were treated with energy set at 1.5 W. The optical fiber was introduced 1 mm short of the apex and recessed in helicoidal movements at a speed of approximately 2 mm/s for 5 s, repeated 4 times at intervals of 10 s, between each one to avoid temperature change. In group C, the metallic nozzle generating the plasma was inserted into the coronal patency of each root canal for 60 seconds. The plasma device used here comprised of two metal electrodes and was powered by an AC power supply. Vacuum chamber was embedded in the handle unit. This glass tube was covered by PVC insulating tube to prevent plasma leakage, and the end of the tube was left exposed to a thin metal tube inserted in a rubber lid covering the top portion of the PVC tube. Effectively the vacuum glass tube was then in contact only with the metal nozzle and insulated all around. Plasma, which was generated in vacuum glass chamber, was then emitted only through the metallic nozzle.

Groups tested: All procedures were carried out in a laminar flow chamber using sterile instruments. Sterile paper points were introduced to working length for 15 s and allowed to saturate. Following to each sampling, paper points were transferred to tubes containing 1 ml of freshly prepared BHI broth and vortexed for 20 seconds. After 10-fold serial dilutions, aliquots of 0.1 ml were plated onto blood agar plates and incubated at 37°C for 24 h. The colony-forming units (CFUs) grown were counted and recorded.

Statistical Analysis: Descriptive and inferential statistical analyses were carried out in the present study. Results on continuous measurements were presented on Mean ± SD. Level of significance was fixed at p=0.05 and any value less than or equal to 0.05 was considered to be statistically significant. Analysis of variance (ANOVA) was used to find the significance of study parameters between three groups followed by Tukey's post hoc analysis.

The Statistical software IBM SPSS statistics 20.0 (IBM Corporation, Armonk, NY, USA) was used for the analyses of the data and Microsoft Word and Excel were used to generate graphs, tables etc**.**

CFUs seen in: NaOCl group **Laser group** **Plasma group**

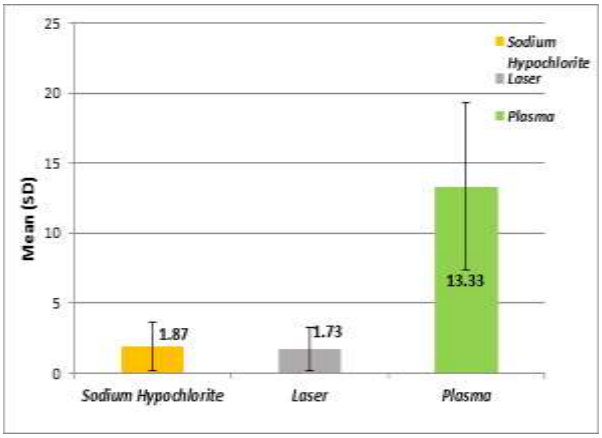
Results

The total microbial count was estimated using a digital colony counter. Count per milliliter of diluted broth was calculated and multiplied by dilution factor.

Table 1: Comparison of the colony forming units in terms of {Mean (SD)} among all the 3 groups using ANOVA test

Treatment Group	N	Mean	Std. Deviation	F value	P value
Sodium Hypochlorite	15	1.87	1.727	49.184	<0.001**
Laser	15	1.73	1.534		
Plasma	15	13.33	5.936		
Total	45	5.64	6.568		

($p< 0.05$ - Significant*, $p < 0.001$ - Highly significant**)



Discussion

The reduction and elimination of micro-organisms are of utmost importance towards achieving successful endodontic therapy. To achieve this goal, mechanical preparation, irrigation, disinfection and obturation of the root canal system is necessary.[14,15] According to studies, microorganisms penetrate deep into anatomic regions such as lateral canals, apical ramifications, isthmuses, dentinal tubules and are also present in smear layer.[15,16] Mechanical debridement alone does not result in total or permanent reduction of bacteria.[16] Anti-microbial agents have been recommended as an adjunct to mechanical instrumentation to reduce the number of micro-organisms.[17,18]

It is important to validate the bactericidal action of different disinfection methods using a resistant microorganism such as E. faecalis. E. faecalis has an ability to grow in high salt concentrations, a wide temperature range, has tolerance to a broad pH range, as well as resists the intracanal procedures.[19] Studies have also revealed that E. faecalis has the ability to penetrate far into dentinal tubules and form biofilms in root canals, and thus escapes from the action of endodontic instruments and irrigants used during chemo-mechanical preparation.[20] E. faecalis was chosen for this present investigation, as it has been the most prevalent bacterial strain in the failed root canal system. Also it has been used for evaluation of the antibactericidal effects of several irrigation solutions and various devices like laser and plasma.

In our study, sodium hypochlorite was used as an anti-microbial irrigant which successfully eradicates E. faecalis. NaOCl is an effective antimicrobial agent, good lubricant and an excellent organic solvent. However, it is highly irritating to the periapical tissues, especially at high concentrations.[18] In cases of extravasation, 5.25% NaOCl, due to its high cytotoxicity, can cause sequelae such as pain, swelling, bruising and numbness. Studies have compared the antimicrobial activity of 2.5% and 5% concentration NaOCl against E. faecalis and found no significant differences between the concentrations tested.[21] The 2.5% NaOCl proved to be a better solution than the others, because it has greater effectiveness than 0.5% and 1% concentrations and has lower cytotoxicity than the 5.25% concentration.[22] The 2.5% NaOCl is capable to inhibit 100% of the Enterococcus faecalis in 5 minutes.[23]

Various studies show depth of penetration of irrigants to be limited to 100 µm, whereas E. faecalis is known to penetrate to a depth of 600-1000 µm.[24,25] Bacteria deep in dentinal tubules are apparently protected from instrumentation and irrigation, making their removal or eradication difficult. In such cases, besides conventional irrigants, adjuncts like laser and plasma prove to be valuable in elimination of bacteria.

Diode laser was used in this study because of it desirable properties such as high penetration depth into the dentinal tubules and proper antibacterial effect.[26,27] The laser radiation may be transmitted through quartz optical fibers, a property that could facilitate introducing laser light around canal curvatures and irregularities.[28] The fine diameters of optic fibers (200-320 µm) enable effective delivery of laser light to the root canal to help with reduction of bacterial contamination. The antibacterial effect observed reaches over 1 mm deep into the dentin, surpassing the effective range of chemical disinfectants, such as NaOCl and displaying moderate effectiveness against E. faecalis even in the deeper layers of dentin.[29] The diode laser used in this study was iLase (Biolase, California, USA) with an endodontic tip (ezTipEndo, 20mm/200µm).

Recent development of nonthermal, atmospheric-pressure plasmas that can enter the root canal of teeth that have been drilled and cleaned has made it possible to use the plasma to remove the microorganisms associated with infected root canals.[30] Plasma is a novel antimicrobial intervention, and plasma devices have shown to kill a higher proportion of bacteria than conventional non-thermal methods such as UV sterilization.[31] Also, plasma can affect not only the contacted point but also the area around it. The plasma device used in this study was self-designed.

All the three groups in this study showed effective antimicrobial activity against E. faecalis. However, the laser and NaOCl groups showed statistically significant decrease in the E. faecalis count compared to the plasma group. On comparing the antimicrobial efficacy of the diode laser against 2.5% NaOCl, diode laser proved to be slightly better than the latter. However, the difference was statistically insignificant, leading to the conclusion that laser proved to be equally efficacious as a root canal disinfectant as sodium hypochlorite. The superior bactericidal effect of diode laser irradiation could be attributed to its greater depth of penetration (up to 1000 µm into dentinal tubules) when compared to the penetration power of chemical disinfectants,

which is limited to 100 μm.[24,25] A study by S. Kumar et al also showed similar results and concluded that diode laser alone and diode laser with sodium hypochlorite showed complete elimination of E. faecalis from the root canal.[32] This can be explained by the broad antimicrobial spectrum of sodium hypochlorite, demonstrated by earlier studies[33] as well as to the protein denaturation and photothermal action provided by high-power diode laser over the bacterial cell.[34]

In the present study, significant difference was found between sodium hypochlorite and plasma group, and laser and plasma group. The results observed here could be because the plasma device used in this study is a self-designed one and no experimental applications had been done before this to check its anti-microbial efficacy. Also the procedure is highly sensitive and there is a room for operative errors. Various studies show effectiveness of plasma to eradicate E. faecalis.[11] Pan et al also concluded that the cold plasma has a high efficiency in disinfecting the Enterococcus faecalis biofilms in vitro in root canal treatment.[35] The results of this study also indicate that plasma is effective in eradicating E. faecalis. The highest concentration of plasma energy occurs 5–6 mm beyond the plasma needle, hence plasma seems to be effective in deep parts of dentin.[36] Thus, it can be concluded that plasma can also eliminate the bacteria but it warrants further investigation.

It should be noted, however, that none of the treatment regimens were able to render the canals free of bacteria in all samples. Microbial communities in vivo are quite resistant to and difficult to eradicate. The testing of anti-microbial agents against bacterial biofilms is yet to be standardized and no in vitro method accurately reflects the conditions under which microorganisms grow in vivo.

Conclusion

Within the limitations of this in vitro study, the following conclusions were made:

1. Significant bacterial reduction was observed in all the three groups.
2. Plasma microjet exhibited least antimicrobial activity as compared with diode laser and sodium hypochlorite.
3. Diode laser and sodium hypochlorite proved to be equally efficacious in their anti-microbial properties against E. faecalis.
4. There was a statistically significant difference observed among the three groups.

References

1. Chavez de Paz LE. Redefining the persistent infection in root canals: possible role of biofilm communities. J Endod 2007;33:652–62.
2. J E Sundqvist G, Figdor D, Persson S, Sjögren U. Microbiologic analysis of teeth with failed endodontic treatment and the outcome of conservative re-treatment. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1998;85:86–93.
3. Distel JW, Hatton JF, Gillespie MJ. Biofilm formation in medicated root canals. J Endod 2002;28:725-7.
4. Spratt DA, Pratten J, Wilson M, Gulabivala K. An in vitro evaluation of the antimicrobial efficacy of irrigants on biofilms of root canal isolates. Int Endod J 2001;34:300-7.
5. Bloomfield SF, Miles GA. The antibacterial properties of sodium dichloroisocyanurate and sodium hypochlorite formulations. Journal of Applied Bacteriology 1979;46:65–73.
6. Garcia F, Murray PE, Garcia-Godoy F, Namerow KN. Effect of aquatine endodontic cleanser on smear layer removal in the root canals of ex vivo human teeth. J Appl Oral Sci 2010;18:403–408.
7. Smitha T, Chaitanya Babu N. Plasma in Dentistry: An update. IJDA. 2010;2:210-14.
8. Martin M. From distant stars to dental chairs- plasmas may promise pain-free and durable restorations. AGD Impact 2009;37(7):46.
9. Jiang C, Chen M, Schaudinn C, Gorur A, Vernier PT, Costerton W, Jaramillo D, Sedghizadeh PP et al. Pulsed atmospheric-pressure cold plasma for endodontic disinfection. IEEE Trans Plasma Sci 2009;37:1190–95.
10. Yamazaki H, Ohshima T, Tsubota Y, Yamaguchi H, Jayawardena JA, Nishimura Y. Microbicidal activities of low frequency atmospheric pressure plasma jets on oral pathogens. Dent Mat J 2011;30:384–391.
11. Lu X, Cao Y, Yang P, Xiong Q, Xiong Z, Xian Y and Pan Y. An RC plasma device for sterilization of root canal of teeth. IEEE Trans Plasma Sci 2009;37:668–673.
12. Rooney J, Middsm, Leeming J. A laboratory investigation of the bacteriocidal effect of a Nd: YAG laser. Br Dent J 1994; 176:61-4.
13. Souza EB, Amorim CV, Lage-Mrques JL. Effect of diode laser irradiation on the apical sealing of MTA. Braz Oral Res 2006; 20:231-4.
14. Lekshmy DS, Kamath PM. Antimicrobial efficacy of 0.2% chlorhexidine and 2% sodium hypochlorite as root canal irrigants: An in vivo study. J Endodontol 2001;13:57-62.
15. Stuart CH, Schwartz SA, Beeson TJ, Owatz CB. Enterococcus faecalis: its role in root canal treatment failure and current concepts in retreatment. J Endod 2006;32:93-98.
16. Abdullah M, Ling Y, Gulabivala K, Moles DR, Spratt DA. Susceptibility of two Enterococcus faecalis phenotypes to root canal medications. J Endod 2005;31(1):30-36.
17. Gomes BPFA, Lilley JD, Drucker DB. Variations in the susceptibilities of components of the endodontic microflora to biomechanical procedures. Int Endod J 1996;29:233-41.
18. Ercan E, Özekinci T, Atakul F, Kadri Gül. Antibacterial Activity of 2% Chlorhexidine Gluconate and 5.25% Sodium Hypochlorite in Infected Root Canal: In Vivo Study. JOE 2004;30(2):84-87.
19. Tendolkar PM, Baghdayan AS, Shankar N. Pathogenic enterococci: new developments in the 21st century. Cell Mol Life Sci. 2003;60(12):2622-36.
20. Card SJ, Sigurdsson A, Orstavik D and Trope M. The Effectiveness of Increased Apical Enlargement in Reducing Intracanal Bacteria. J Endod 2002;28(11):779-783.
21. Sevinç Aktemur Türker, Mehtap Hülya Aslan, Sevilay Karahan, Emel Uzunoğlu. Effect of temperature, concentration, and contact time of sodium hypochlorite

on Enterococcus faecalis. Clinical Dentistry and Research 2015;39(2):69-73.
22. Mohammadi Z. Sodium hypochlorite in endodontics: an update review. Int Dent J 2008;58:329-341.
23. Radcliffe CEL, Potouridou R, Qureshi N, Habahbeh A, Qualtrough H, Worthington DB. Antimicrobial activity of varying concentrations of sodium hypochlorite on the endodontic microorganisms Actinomyces israelii, A. naeslundii, Candida albicans and Enterococcus faecalis. Int Endod J. 2004;37(7):438-46.
24. George S, Kishen A, Song KP. The role of environmental changes on monospecies biofilm formation on Root canal wall by Enterococcus faecalis. J Endod 2005;31:867-72.
25. Moritz A, Beer F, Goharkhay K, Schoop U. Laser Supported Root Canal Sterilization. Oral Laser Application. 1st ed. Chicago, IL: Quintessence Publishing; 2006:254-77.
26. Gutknecht N, Van Gogswaardt D, Conrads G, Apel C, Schubert C, Lampert F. Diode laser irradiation and its bactericidal effect in root canal wall dentin. J Clin Laser Med Surg 2000;18(2):57-60.
27. Moritz A, Gutknecht N, Goharkhay K, Schoop U, Wernisch J, Sperr W. In vitro irradiation of infected root canals with a diode laser: results of microbiologic, infrared spectrometric, and stain penetration examinations. Quintessence Int. 1997;28(3):205-9.
28. Stabholz A, Sahar-Helft S, Moshonov J. The use of lasers for cleaning and disinfecting of the root canal system. Alpha Omegan 2008;101(4):195-201.
29. 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.
30. Jiang, M. T. Chen, A. Gorur et al. Nanosecond pulsed plasma dental probe. Plasma Processes and Polymers 2009; 6(8):479–483.
31. Kim GC, Kim GJ, Park SR, Jeon SM, Seo HJ, Iza F and Lee JK. Air plasma coupled with antibody-conjugated nanoparticles: a new weapon against cancer. J Phys D: Appl Phys 2009;42:032005.
32. S Kumar, Sivakumar Kailasam, Sebeena Mathew, Harikaran, Karthick, Boopathi. Comparative evaluation of antimicrobial efficiency of diode laser, sodium hypochlorite and their synergistic effect against Enterococccus faecalis contaminated root canals- an in vitro study. Asian Pac J Health Sci 2014;1(3):244-249.
33. Krause TA, Liewehr FR, Hahn CL. The antimicrobial effect of MTAD, sodium hypochlorite, doxycycline, and citric acid on Enterococcus faecalis. J Endod 2007;33:28-30.
34. Gutknecht N, Moritz A, Conrads G, Sievert T, Lampert F. Bactericidal effect of the Nd: YAG laser in vitro root canal. J Clin Laser Med Surg 1996;14:77-80.
35. Pan J, Sun K, Liang Y, Sun P, Yang X, Wang J, Zhang J, Zhu W, Fang J, Becker KH. Cold Plasma therapy of a tooth root canal infected with Enterococcus faecalis biofilms in vitro. J Endod. 2013;39(1):105-10.
36. Kim SJ, Chung TH and Bae SH. Striation and plasma bullet propagation in an atmospheric pressure plasma jet. Phys Plasmas 2010;17,053504.