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Original Article

Antibacterial effects of ER: YAG-PIPS, ER, CR: YSGG laser and conventional irrigation on enterococcus faecalis and candida albicans

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Abstract

Aim: Root canal treatment aims to disinfect root canal system from microorganisms. The limitations of contemporary disinfectants require investigation of new techniques. The aim of the present study is to compare the antibacterial efficiency of Er,Cr:YSGG [RFT] and Er:YAG [PIPS] lasers on Enterococcus faecalis [E. faecalis] and Candida albicans [C. albicans] to that of NaOCl.

Material and Methods: The present study included 140 single rooted human mandibular premolars. After preparing and sterilizing, the samples were divided into two groups. Root canals in group 1 were contaminated with E. faecalis while root canals in group 2 were contaminated with C. albicans. The root canal of each sample except negative control group was inoculated with 105cfu/ml of microorganisms in order to achieve standardization. Following incubation, all samples were randomly divided into 10 subgroups; Group 1A: Er,Cr:YSGG laser group. Group 1B: Er:YAG-PIPS laser. Group 1C: 5% NaOCl. Group 1D: positive control group. Group 1E: negative control group. Group 2A: Er,Cr:YSGG laser group. Group 2B: Er:YAG laser. Group 2C: 5% NaOCl. Group 2D: positive control group. Group 2E: negative control group. After disinfection process, the culture of each sample was tested to determine cfu/ml values for before incubation and after 4 week incubation.

Results: : Er:YAG and NaOCl groups lead to significant microorganism reduction [$p<0.05$]. In Er,Cr:YSGG group, reduction was not significant [$p>0.05$].

Conclusion: The use of Er:YAG-PIPS laser with NaOCl increased the success rate in the elimination of E.faecalis and C.albicanss.

Keywords: Candida albicans; Er,Cr:YSGG laser; Er:YAG-PIPS laser; enterococcus faecalis; NaOCl

INTRODUCTION

The main reason for the failure of endodontic treatment is the virulence and number of residual bacteria which gain access to periapical tissues (1). Particularly, Enterococcus faecalis (E. faecalis) and Candida albicans (C. albicans) which are highly resistant to root canal disinfection methods have been frequently detected in failed root canals (2, 3). Although, disinfection of main canals can be easily achieved with routine chemo-mechanical disinfection methods, dentinal tubules, lateral canals and apical delta may harbor remaining microorganisms (4). Several irrigation activation methods have been employed in order to overcome these limitations of current root canal disinfectants [especially sodium hypochlorite (NaOCl)]. Thus, both the efficiency and the penetration depth of these solutions have been enhanced.

Lasers are contemporary activation methods providing the elimination of bacteria present in non-reachable areas (5). Particularly, Er,Cr:YSGG lasers equipped with radial firing tip (RFT) and Er:YAG lasers equipped with photon-induced photoacoustic streaming (PIPS) tips were manufactured for the activation of intra-canal disinfectants (6). In Erbium lasers, energy generated is absorbed by the subsequent solution leading to quick (in 1 μs) vaporization. Fast expansion and shrinkage of the bubbles following vaporization lead to supersonic and sonic waves in intra-canal disinfection solution. These shock-waves generated in root canals favor the

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bactericidal effect of intra-canal solution (7).

In such a similar manner, PIPS technique results in photoacoustic streaming in root-canal disinfectants and by this way provides a better cleansing of root canals (8).

The aim of the present study is to compare the antibacterial potential of PIPS attached Er:YAG laser and RFT attached Er,Cr:YSGG lasers with conventional root canal irrigation against E. faecalis and C.albicans.

MATERIAL and METHODS

The present study was approved by the local ethical committee of Gaziantep University (2016/433) and included 140 intact, mature, single straight rooted mandibular premolars. The presence of only one canal and absence of calcification was confirmed with radiographs. All teeth were disinfected in thymol and later kept in 0.9% saline solution until they were used. Root surfaces were examined under stereomicroscope (Leica Microsystems, Wetzlar, Germany) with a magnification of x20 and roots representing any crack or craze lines were excluded. Root surfaces of included samples were cleaned with curettes. All teeth were decoronated until 13 mm long roots were obtained. A size 10 K-file was inserted in root canals until its tip is visible at the apex. Working length was determined as 1 mm short of this point. Root canals were prepared with Reciproc R40 instrument (VDW, Munich, Germany) attached to endodontic motor (Dentsply, Maillefer, Switzerland) at special settings of Reciproc. Root canals were rinsed with saline solution during preparation and a final irrigation with 20 ml 17% EDTA (Werax, Spot diş Deposu AS, İzmir, Turkey) in a total of 2 minutes duration. Root canals were dried with absorbent paper points (Pearl Endo, Gyonggi-Do, Korea). External root surfaces were coated with 2 coats of nail varnish and apices were sealed with composite resin to avoid the extrusion of inoculated bacteria.

Roots were fixed in Eppendorf tubes by using self-curing acrylic (Meliodent, Bayer Dental, Leverkusen, Germany) and taps were tightly closed. Subsequently, all root canals were rinsed with 5 ml 17% EDTA for 1 minute, 5 ml saline for 1 minute and dried with paper-points (Pearl Endo, Gyonggi-Do, Korea). All samples were sterilized with Anprolen Gaz (Caps, Chine) at 74oC cultivation was achieved with National Collection of Type Cultures, E. faecalis (E. faecalis ATCC 29212) standard bacteria at 5% sheep blood agar and C. albicans (C. albicans ATCC 90028) standard fungi at Saboraud Dextrose Agar (SDA, Bec- ton Dickinson, USA). All were incubated at 37oC for 24 hours.

The suspension was adjusted to a McFarland standard number 0.5 to ensure that the concentration of bacteria was 1.5x105cfu\ml. Each canal was filled with 10 µl either E. faecalis or C. albicans suspension according to the relevant group (Figure 1 a,b):

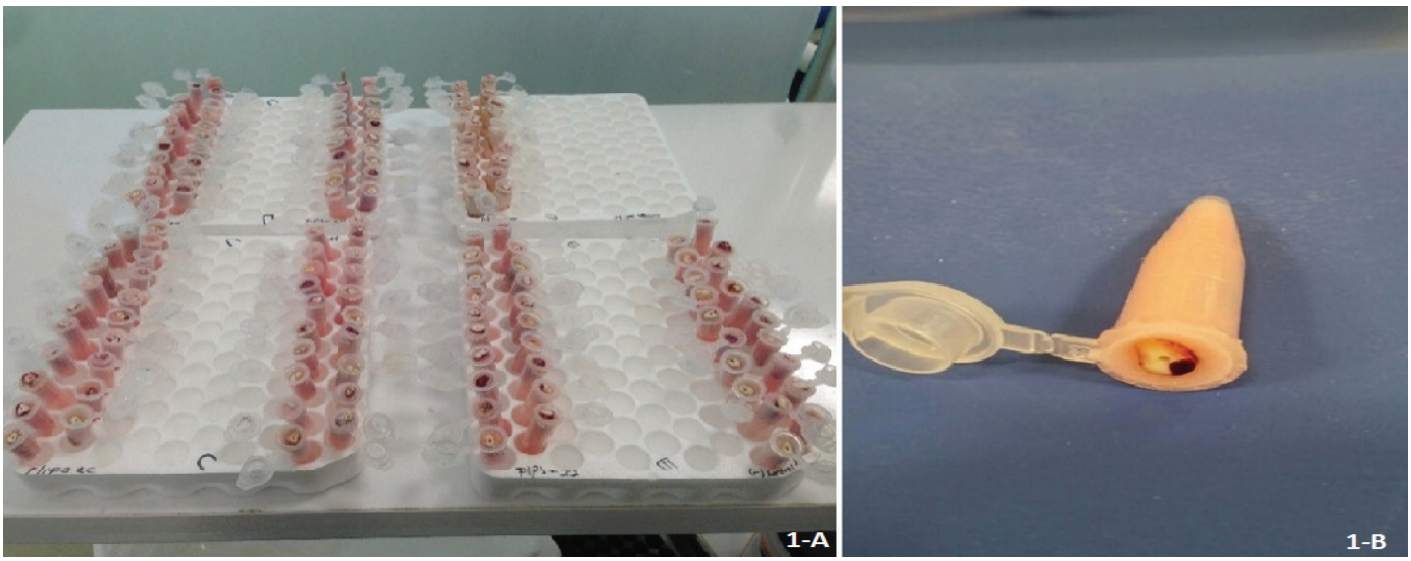

Figure 1 a.b. Prepared and inoculated samples

Groups 1: E. faecalis infected groups:

Group 1A: Er,Cr:YSGG laser group (n = 20)
Group 1B: Er:YAG – PIPS laser group (n = 20)
Group 1C: Conventional irrigation with 5% NaOCl (n = 20)
Group 1D: Positive control (n = 5)
Group 1E: Negative control (n = 5)

Groups 2: C. albicans infected groups:

Group 2A: Er,Cr:YSGG laser group (n = 20)
Group 2B: Er:YAG – PIPS laser group (n = 20)
Group 2C: Conventional irrigation with 5% NaOCl (n = 20)
Group 2D: Positive control (n = 5)
Group 2E: Negative control (n = 5)

Disinfection with Er,Cr:YSGG

In groups 1A and 2A, 2940 nm wavelength Er,Cr:YSGG laser (WaterLase* iPlus, Biolase, Irvine, CA, USA) was set to 2 W output power, 20 Hz repetition rate, 25 mj pulse energy, 25% water and 35% air. RFT-2 fiber tip with 200 µm diameter and 21 mm long attached to the hand piece was inserted 2 mm short of working length and withdrawn 1 mm/second during 12 seconds (Figure 2). This was repeated 4 times with 10 second break between each application. During irradiation, it was attended to touch all root canal walls with a circular motion. No irrigation was performed following irradiation.

Disinfection with Er:YAG – PIPS laser

In groups 1B and 2B groups, Er:YAG laser (Fidelis AT, Fotona, Ljubljana, Slovenia) was set to 0.8 W output power, 20 Hz repetition rate, 40 mj pulse energy. Irradiation was performed simultaneously with 5% NaOCl irrigation by placing the PIPS tip into 3 mm deeper from canal orifices for 5 times* 5 seconds (Totally 25 seconds) (Figure 3). For each canal, 7 ml NaOCl was used and the solution was delivered into the canals with double side-vented 31-gauge endodontic irrigation needles (i-Tips, i dental, Siauliai, Lithuania) positioned at a depth of 2 mm shorter from working length.

Figure 2. Disinfection with Er,Cr:YSGG laser

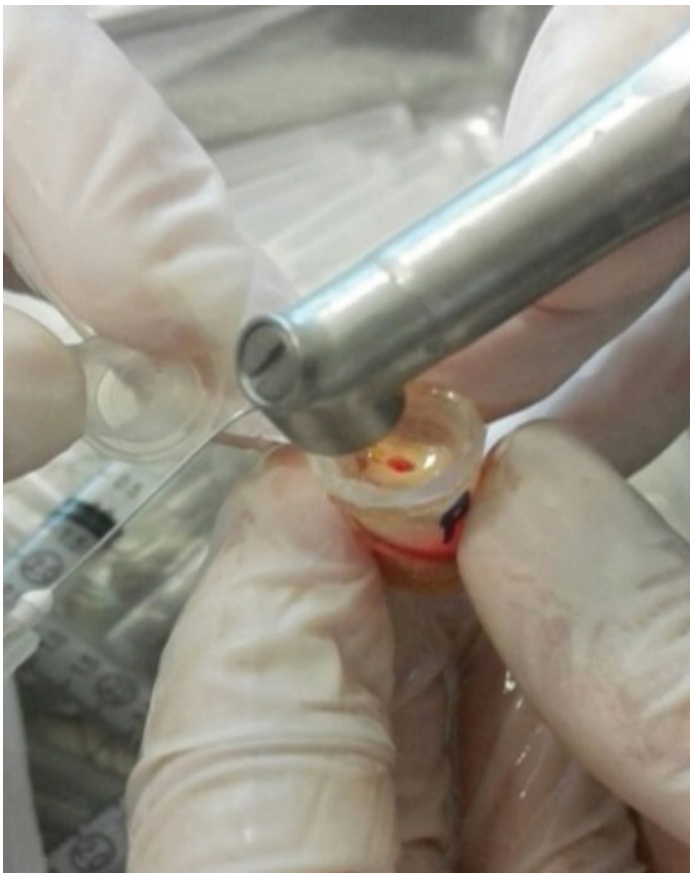

Figure 3. Disinfection with Er:YAG – PIPS laser

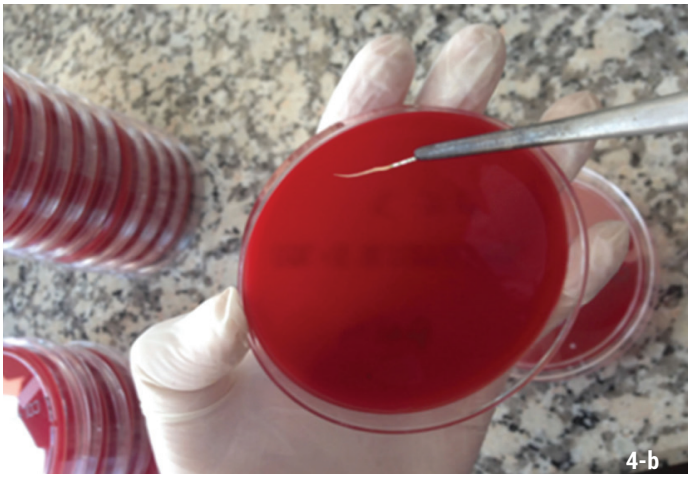

Figure 4 a.b. Bacterial counting following disinfection process.

Sodium Hypochlorite (NaOCl) Groups

For groups 1C and 2C groups, each canal was rinsed with 7 ml 5% NaOCl by using double side-vented 31-gauge endodontic irrigation needles positioned at a depth of 2 mm shorter from working length for 2 minutes.

Positive Control

In groups 1D and 2D, inoculation was performed but no disinfection method was applied.

Negative Control

Groups 1E and 2E were sterilized but no inoculation was performed.

Following disinfection processes, absorbent paper points were placed into the canals for 1 minute in order to take samples. These paper points were left in broth for incubation. Furthermore, samples were left for another 4 weeks by refreshing broth in each 4 days and then a second cultivation was performed. By this way, we aimed to evaluate the residual bacteria following both immediate after and 4 weeks after the relevant disinfection process. After each application, the taps of the tubes were tightly closed.

Microbial counting

Following disinfection procedures, microbial samples were obtained by keeping saline absorbed paper points in root canals for 1 minute. After that these paper points were swabbed into the broth containing plates. All plates were closed tightly. First microbial counting was performed 24 hours later. The number of cfu/ml was calculated for each samples and its corresponding plate. Then, samples were incubated for 4 weeks by adding fresh Brain Heart Broth into root canals in every 3 days. A second counting was performed at the end of 4 weeks (Figure 4 a,b). Incubation media was 37^{0}C. All bacterial counting was recorded in cfu/ml for each specimen.

Statistical Analysis

Prior to the study, the number of samples in each group was determined with Power Analysis. The distribution of data was analyzed with Shaphiro-Wilk test. Because of abnormal distribution of the data, Kruskall Wallis and Dunn multi comparison tests were used. Statistical analyze was performed by using SPSS version 22.0. The significance was set to <0.05.

RESULTS

Results of E. faecalis infected canals

The amount of residual microorganisms in E. faecalis infected groups after disinfection procedures and 4 weeks later was represented in Table 1. All disinfection methods decreased intra-canal E. faecalis significantly. The Er:YAG- PIPS group was found to be the most effective. However, it was not significantly superior to NaOCl ($p > 0.05$) Er,Cr:YSGG was significantly ineffective compared to Er:YAG – PIPS ($p < 0.05$).

Following 4 weeks; Er:YAG was the best while Er,Cr:YSGG was the worst. The difference among the 3 groups were statistically significant ($p <0.05$).

Table 1. The mean and standard deviation values of the E. faecalis amount in the experimental groups immediately and 4 weeks after the disinfection process

Bacteria	Groups	N	Initial*	N	4 weeks*	P (In-group)
E. faecalis	Er,Cr:YSGG	20	5.25±7.46 ǂ¥	20	48.65±20.99 ǂ¥	0.001 ⸗
	Er:YAG	20	0.6±2.25 ǂ§	20	3.55±7.43 ǂ§	0.001 ⸗
	NaOCl	20	2.21±4.98 ǂ	20	9.1±19.12 ǂ§	0.176
	Positive	5	100±0	5	100±0	1
	Negative	5	0±0 ǂ	5	0±0	1
P (between groups)			0.001 ⸗			0.001 ⸗

* Average cfu ± standard deviation
⸗Statistically significant at 0.05 level
ǂ According to positive control p<0.05
§ According to Er,Cr:YSGG laser p<005
¥ According to Er:YAG laser p<0.05

Results of C.albicans infected canals

The mean amount of residual C. albicans immediately after canal disinfection procedure and 4 weeks later was represented in Table 2. All disinfection methods lead to a significant decrease in the amount of C. albicans. The most effective method was conventional NaOCl irrigation while the least effective one was Er,Cr:YSGG just after disinfection. The difference of Er:YAG-PIPS with both was not statistically significant ($p>0.05$).

Following 4 weeks, NaOCl irrigation and Er:YAG-PIPS groups were sig- nificantly superior to Er,Cr;YSGG laser ($p<0.05$) while similar to each other ($p>0.05$).

Table 2. The mean and standard deviation values of the C.albicans amount in the experimental groups immediately and 4 weeks after the disinfection process

Bacteria	Groups	N	Initial*	N	4 weeks*	P(In-group)
C.albians	Er,Cr:YSGG	20	7.2±7.85 ǂ	20	73.15±34.04	0.001 ⸗
	Er:YAG	20	3.6±8.09 ǂ	20	8.15±13.13 ǂ §	0.306
	NaOCl	20	0.5±2.23 ǂ §	20	0.05±0.22 ǂ §	0.655
	Positive	5	100±0	5	100±0	1
	Negative	5	0±0 ǂ	5	0±0 ǂ §	1
P (between groups)			0.001 ⸗			0.001 ⸗

* Average cfu ± standard deviation
⸗Statistically significant at 0.05 level
ǂ According to positive control p<0.05
§ According to Er,Cr:YSGG laser p<0.05

DISCUSSION

E. faecalis is able to survive even in high pH environment, form biofilm and resist to intra-canal disinfectants (9). C. albicans is another endodontic pathogen with high affinity to dentine and the capability of tubular invasion (10). They are the most common microorganisms isolated from failed root canals. In particular, E. faecalis can survive in deeper parts of dentinal tubules and C. albicans can

strictly adhere to root dentin (9). Due to these reasons, these 2 species of endodontic pathogens are considered as main microbial factors of failure, the present study included E. faecalis and C. albicans.

Previous studies reported different incubation periods ranging between 24 hours and 4 weeks for E. faecalis (11,12) and 2-28 days for C. albicans (13-15). In the present study, incubation was performed for 4 weeks to provide extended tubular invasion.

The efficiency of laser aided disinfection depends on the heat generated during irradiation. In PIPS technique which is a modification of Er:YAG laser, photo acoustic streaming effect provides a further cleansing efficiency for canal disinfection. This may explain why PIPS application in which NaOCl irrigation lasted for only 25 seconds was equal to NaOCl alone group (NaOCl irrigation for 2 minutes) because it is evident that increasing the heat of NaOCl in root canals enhances its efficiency and reduces the required time for intra-canal application. Furthermore our results are in accordance with Wang et al. (16) who found Er,Cr:YSGG laser as lesser effective than conventional irrigation with 2.5% NaOCl. These results highlight the importance of the term "laser aided disinfection" which means laser systems may improve the efficiency of intra-canal irrigants but not effective alone. On the other hand, Onay et al. (17) found that Er,Cr:YSGG laser is effective when it is used as a conjunct to NaOCl. These results are true for each 2 bacterial species just after disinfection. Despite the difference is not significant. NaOCl is slight superior to PIPS for C. albicans which is probably related to the duration of irrigation. In other words, because C. albicans has a strict affinity to root dentine, time of application is more important than activation method for this microorganism. Furthermore, following 4 weeks, both microorganisms increased significantly in Er,Cr:YSGG laser group. This is presumably related to the inability of this system to invade and cleanse the deep parts of dentinal tubules.

Conversely, Gordon et al. (18) found Er,Cr:YSGG laser more effective than NaOCl. However that study was performed on dentin discs which cannot totally simulate intra-canal conditions. Also, Kasic et al. (19) found Er, Cr:YSGG laser superior to Er:YAG laser contrary to our findings. However. they used Er:YAG laser not in PIPS form and their incubation period is 7 days which is lesser than us. Thus we assume that Er:YAG laser is more effective in PIPS form which causes micro-explosions of intra-canal NaOCl. Furthermore, keeping microorganism in root canals for longer incubation periods seems more reliable because of providing more tubular invasion. In the study of Korkut et al. (20), Er:YAG-PIPS laser found to be superior to conventional irrigation on E. faecalis. Although the difference in our study is not significant, PIPS is reduced slightly more E. faecalis compared to NaOCl alone.

Franzen et al. (21) evaluated the antibacterial efficiency of Er,Cr:YSGG laser on 100 µm and 1000 µm dentine slices and found that its effectiveness significantly decreased with increasing depth in accordance with the present study. The present study performed intra-canal cultivation both just after and 4 weeks later disinfection to evaluate the re-invasion of bacteria from tubules to root canals and by this way pointed the importance of lateral extension of the antibacterial effect of irrigation solutions throughout tubules. lateral canals and other irregularities which is important for long-term success.

CONCLUSION

Within the limitations of the present study, it can be concluded that NaOCl activated with PIPS seems a promising technique for intra-canal disinfection. For C.albicans, duration of irrigant application is more important than activation. Laser systems are supportive to conventional irrigation materials in case of combine use.

Competing interests: The authors declare that they have no conflict of interest.
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Ethical approval: The present study was approved by the local ethical committee of Gaziantep University (2016/433).

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