IEJ
Iranian Endodontic Journal

Antibacterial Effect of Photodynamic Therapy on Root Canal Disinfection Combined with Different Irrigation Protocols

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ARTICLE INFO

Article Type: **Original Article**

Received: 13 Dec 2019
Revised: 18 Feb 2020
Accepted: 01 Mar 2020
Doi: *10.22037/iej.v15i2.27801*

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ABSTRACT

Introduction: Photodynamic therapy (PDT) is an emerging alternative therapy to conventional endodontic treatment to optimize bacterial elimination. The aim of this study was to evaluate the *in vitro* antibacterial effect of PDT combined with different irrigation protocols on root canals inoculated with *Enterococcus* (*E.*) *faecalis*. **Methods and Materials:** Ninety uni-radicular human premolars were prepared and contaminated with *E. faecalis* for 4 days. Teeth were randomly divided into six groups: positive control group (C+) consisted of conventional needle irrigation with 2.5% sodium hypochlorite (NaOCl); negative control group (C-) consisted of no treatment after contamination; PDT group as treated with 0.005% methylene blue and diode laser irradiation for 90 sec at wavelength of 660 nm, energy of 9 Joules, power of 100 mW; the fourth group consisted of NaOCl+PDT, the fifth group were treated with passive ultrasonic irrigation (PUI) with NaOCl+PDT (PUI+PDT); and the final group were treated with XP Endo Finisher with NaOCl+PDT (XP Endo+PDT). The contents of the root canals were collected with sterile absorbent paper points at two times: before and 24 h after decontamination protocols. The number of colony-forming units (CFU) was determined for each root canal. ANOVA and the Tukey test were used, with significance set at 5% ($P<0.05$). **Results:** The inhibition percentage ranged from 10.72 (C-) to 100% (XP Endo+PDT), with CFU/mL counts differing among all protocols tested ($P<0.05$). The different protocols significantly influenced bacterial inhibition ($P<0.05$). However, the XP Endo+PDT protocol resulted in the highest inhibition percentage (100%), followed by NaOCl+PDT (65.85%). **Conclusions:** PDT combined with different final irrigation protocols was more effective in inhibiting *E. faecalis* growth than photodynamic therapy alone. XP Endo was the best irrigation protocol to eradicate this microorganism.

Keywords: Biofilm; Endodontics; *Enterococcus faecalis*; Photodynamic Therapy

Introduction

The primary goal of endodontic treatment of necrotized teeth is to eliminate or reduce bacterial populations inside the root canal to levels that allow the healing of the periradicular tissue [1]. The anatomical complexity of the root canal system makes complete bacterial debridement almost impossible [2]. Even if conventional methods are replaced by modern techniques that use nickel-titanium files, more than 35% of the root canal surface may remain uninstrumented after non-surgical endodontic treatment [3].

Among residual bacteria, *Enterococcus (E.) faecalis* is the most common species found in treated canals and has been indicated as the main pathogen involved in endodontic treatment failure due to its malevolent ability to survive under difficult environmental conditions [4, 5].

In an attempt to overcome the limitations of conventional endodontic treatment, new adjuvant strategies have been introduced to reduce or eliminate microorganisms, increasing the success rate of this treatment [1]. Among these strategies, photodynamic therapy (PDT) [5-8] passive ultrasonic irrigation

(PUI) [8, 9] and XP Endo Finisher system (FKG Dentaire, La Chaux-de-Fonds, Switzerland) [10, 11] have been associated with improved cleaning and disinfection of the root canal system.

PDT had emerged as an adjunct to conventional endodontic treatment to optimize bacterial elimination [12]. This technique consists of the application of non-toxic photosensitizers that are activated by light at specific wavelength in the presence of oxygen. The energy transfer from the activated photosensitizer to available oxygen results in the formation of toxic oxygen species, known as singlet oxygen and free radicals [4]. The cell wall and membrane of microbial cells are the main targets of the photodynamic process, but DNA damage may also occur [4].

In endodontics, photosensitizers derived from phenothiazine salts have been widely applied in studies on PDT. Methylene blue is one of the main photosensitizers used as a target for microorganisms of the endodontic microbiota [13, 14].

During chemo-mechanical preparation, irrigation solutions act as disinfectant, lubricant and cleaning agents that assist in neutralizing microorganisms, their byproducts tissue remnant and elimination which have been produced by dentin cutting instruments [1]. Irrigation with conventional syringe has been reported to be inefficient in cleaning the more apical portions of the root canal system [15]. Therefore, different final irrigation protocols have been recently introduced in endodontics, promising to optimize root canal disinfection. Irrigation techniques based on some type of agitation, such as passive ultrasonic irrigation, have shown superior results compared to conventional needle irrigation [10] by facilitating penetration of irrigants and removal of the smear layer [16]. The efficiency of PUI is explained by the production of acoustic waves, cavitation and heat generation, which assist in the removal of dentinal tissue remnants and scraping residues [17].

XP Endo Finisher is a new instrument used as final step in the irrigation of root canals. It is a size 25 non-tapered file that respects the original anatomy of the root canal and effectively cleans irregular areas due to its high flexibility and capacity of expansion for three-dimensional adaptation to the root canal [18].

The individual antimicrobial effect of PDT, PUI and XP Endo has been discussed in recent studies [6, 8, 10], but this is the first study to investigate the combination of these protocols for inhibiting *E. faecalis* in root canals. Therefore, the aim of this study was to evaluate the *in vitro* antibacterial effect of PDT combined with different irrigation protocols on root canals inoculated with *E. faecalis*.

Materials and Methods

Selection and standardization of specimens

The present study was approved by the Ethics Research Committee of the Medical Sciences Center, State University of Paraíba, Brasil, under Protocol No. 79027617.8.00005187. This step was the same for all groups. Ninety uni-radicular human premolars were selected. After periapical radiography and careful inspection, teeth with straight canals and fully formed apices, which exhibited no endodontic obturation, cracks or fractures, internal or external resorption or anomalies, were selected. Teeth were transversely cut at the cementum-enamel junction with carborundum disc (KG Sorensen, Barueri, SP, Brazil) to establish a single length of 15 mm for all roots.

The working length was set at 1 mm short of the apical foramen. Specimens were instrumented according to manufacturer recommendations using the Reciproc® R40 rotary system (VDW, Munich, Germany) with tip diameter of 0.40 mm. After each instrument change, irrigation, aspiration and filling with 2.5% sodium hypochlorite were performed with NaviTip® needles (Ultradent, South Jordan, UT, USA) using Ultradent® Irrigation-Aspiration Kit (Ultradent). After instrumentation, the smear layer was removed with 5 mL of 17% EDTA (Biodinâmica, Paraná, Brazil) for 3 min [5] followed by final irrigation with 5 mL NaOCl and 5 mL sterile saline.

After this step, the apex of specimens was sealed with Riva Light Cure® glass ionomer restorative cement (SDI, Victoria, Australia) and root surfaces received a double layer of clear nail polish (Colorama, SP, Brazil). Specimens were transferred to flasks containing brain-heart infusion broth (BHI; Oxoid, Basingstoke, UK) and autoclaved for 20 min at 120°C and 1 atm. Specimens were incubated for 24 h at 37°C to confirm sterility.

Contamination of specimens with Enterococcus faecalis

The contamination protocol was based on method proposed by Andrade *et al.* [19]. Pure *Enterococcus faecalis* cultures (ATCC 29212) were inoculated in tubes with screw caps containing 5 mL BHI. This suspension was mechanically shaken and adjusted in spectrophotometer at 800 nm to concentration equivalent to 1.0 McFarland standard (3.0×10^8 bacteria/mL). The total contamination period was 4 days, with alternating centrifugation cycles. On the first day, the inoculum was prepared. On the first and third days, tubes were centrifuged twice at four speeds (1400, 2000, 3600, and 5600 *g*) for 5 min at 25°C. On the second and fourth days, tubes were centrifuged at 3600 *g* for 5 min at 25°C.

Turbidity was the indicator for bacterial growth in result of bacterial infiltration and this content was submitted to microbiological tests. After the contamination period, canals were dried with absorbent paper points, which were immersed in BHI for 24 h at 37°C for microbial culture and subsequently the material from the canal was analyzed. Contamination with *E. faecalis* was confirmed by the bile-esculin test [20] and tolerance to 6.5% NaCl.

Groups

Specimens were randomly divided into four experimental groups (n=15), positive control group (n=15), and negative control group (n=15), as described below:

C+group (positive control)

Conventional needle irrigation was done in this group. The root canal was irrigated with 1.5 mL of 2.5% sodium hypochlorite for 60 sec using 30-gauge irrigation needle (Ultradent, South Jordan, UT, USA) placed at working length and moved in vertical movements.

C-group (negative control)

Specimens in this group were not treated after contamination with *E. faecalis* cultures.

PDT group

The canal was filled with 0.5 mL of 0.005% methylene blue as photosensitizer (Chimiolux, Aptivalux, Belo Horizonte, MG, Brazil), with pre-irradiation period of 5 min. Subsequently, fiber was inserted into the root canal. Diode laser (Therapy XT, DMC Equipamentos Ltda, São Carlos, SP, Brazil) emitting red light at wavelength (λ) of 660 nm and operating in continuous wave (CW) mode (power of 100 mW, energy of 9 J, and spot size of 0.028 cm^2) was used. Irradiation was performed for 90 sec. The fiber was moved inside the root canal in apical-cervical helical movements. The root canal was irrigated once again with 5 mL sterile saline to remove the photosensitizer.

NaOCl+PDT group

The specimens were irrigated with 1.5 mL of 2.5% NaOCl as described for the C+ group. In addition, PDT was performed using the same parameters as described for the PDT group.

PUI+PDT group

The root canal was irrigated with 1.5 mL of 2.5% NaOCl, followed by ultrasonic agitation with E1 Irrisonic tip (Helse Dental Technology®, SP, Brazil) coupled to ultrasound apparatus (JetSonic BP, Gnatus®, Ribeirão Preto, Brazil) for 60 sec with tip positioned 1.0 mm short of the working length. After this procedure, PDT was performed as described for the PDT group.

XP Endo Finisher+PDT group

After introduction of XP Endo in the working length, the root canal was irrigated with 1.5 mL of 2.5% NaOCl using 30-gauge Ultradent needle. The XP Endo file was used inside the root canal at speed of 800 rpm and torque of 1 Ncm for 60 sec. After this procedure, PDT was performed as described for the PDT group.

After the protocol in experimental groups, root canals were irrigated with sodium thiosulfate to neutralize sodium hypochlorite [5]. Then, they were irrigated with 5 mL of sterile saline.

Sample collection and microbiological analysis

In all groups, samples were collected for microbiological analysis at two times. The first sampling was performed before the application of protocols to confirm contamination of specimens (base line). The second sampling occurred immediately after contamination and microbiological analysis was performed 24 h post incubation of absorbent paper points placed in microtubes (containing brain-heart infusion broth in oven at 37°C) to evaluate microbial reduction.

Samples were collected using three sterile #40 absorbent paper points (Dentsply Maillefer, Ballaigues, Switzerland) after irrigation of canals with 5 mL sterile saline. The paper points used were transferred to tubes containing 1.0 mL BHI and vortexed (IKA® Vortex 3 Genius, Wilmington, NC, USA) for one min. Turbidity of the culture medium was measured in spectrophotometer (Shimadzu® UV mini-1240, Kyoto, Japan) at 540 nm and the number of colony-forming units (CFU) per milliliter was calculated.

Statistical analysis

Data were computed and analyzed using the IBM SPSS Statistics 20.0 software (IBM Corp., Armonk, NY, USA). The root canal disinfection protocol (test groups) was used as the independent variable. The quantity of bacteria observed was the dependent variable (response). The assumption of normal data distribution was confirmed by the Shapiro-Wilk test and parametric tests were also applied. Mixed analysis of variance (ANOVA) was used to detect statistically significant factors or interactions that interfered with the response variable. Multiple comparisons of means were performed using Tukey's HSD post-hoc test [21, 22]. The significance level was set at 5% (P<0.05) in all analyses.

Results

Table 1 shows the comparison of bacterial counts (CFU/mL) at the two times among the different groups. The inhibition percentage ranged from 10.72% (C-) to 100% (XP Endo+PDT). CFU/mL counts were different among all protocols tested (P<0.05). In addition, the different protocols significantly influenced bacterial inhibition (P<0.05). The XP Endo+PDT protocol resulted in the highest bacterial inhibition percentage (100%), followed by NaOCl+PDT (65.85%) (Table 1).

Data also showed the inhibition percentages for the other protocols: NaOCL+PDT (65.85%±4.25%), PUI+PDT (42.51%±6.04%), PUI (39.02±4.68) and PDT (15.15%±4.06%). In addition, the inhibition results for Control+group was 38.00%±4.24%, while for Control-group, inhibition results were 10.72%±5.87%.

Discussion

In this study, the *in vitro* effect of photodynamic therapy associated with different final irrigation protocols for reducing *E. faecalis* contamination in the root canal system was evaluated. The results of this study showed that the different protocols tested all significantly influenced bacterial inhibition, and the XP Endo+PDT group provided the highest bacterial inhibition percentage.

Enterococcus faecalis was used in this study because it is one of the most resistant microorganisms found in endodontic infections, whose prevalence is higher in secondary than in primary infections [23]. Since most endodontic treatment failures are related to microorganisms that persist after conventional endodontic treatment (chemo-mechanical preparation or intracanal medication), new strategies need to be tested in order to maximize *E. faecalis* elimination.

The tooth contamination protocol chosen in this study was based on method proposed by Andrade *et al.* [19], which allows higher and more homogenous proliferation compared to other methods. These factors are essential for antimicrobial studies using *in vitro* models of endodontic infection since they provide more accurate results and avoid false negatives. Although Andrade *et al.* [19] evaluated contamination of dentinal tubules in bovine teeth, satisfactory contamination of the canal of human teeth was obtained in the present study.

The size of the root canal preparation can influence the efficacy of irrigation procedures [24]. In this study, uni-radicular teeth were prepared with #40 instrument (with 0.6 taper). This apical diameter is clinically relevant and allows effective syringe irrigation [25]. In addition, uni-radicular teeth with straight canals were selected to reach comparable bacterial load in all groups, as well as to reduce anatomical variation and achieve standardized sampling.

Table 1. Comparison of bacterial counts (CFU/mL) at two times among different groups

Group	Baseline (CFU/mL)	24 h (CFU/mL)	% Inhibition
C+	52.07 (2.47)^{Ac}	32.29 (2.16)^{Bd}	38.0 (4.24)
C-	43.58 (2.37)^{Aa}	38.91 (1.34)^{Be}	10.72 (5.87)
PDT	51.47 (2.98)^{Ac}	43.67 (1.05)^{Bf}	15.15 (4.06)
NaOCl+PDT	47.76 (2.65)^{Ab}	16.31 (1.61)^{Bb}	65.85 (4.25)
PUI+PDT	47.89 (3.10)^{Ab}	27.53 (1.88)^{Bc}	42.51 (6.04)
XP Endo+PDT	44.16 (2.67)^{Aa}	0.0 (0.0)^{Ba}	100.0 (0.00)

Results are reported as mean (SD). Means followed by different superscript letters differ significantly (P<0.05): uppercase letters compare values in the same row (intragroup comparison: baseline vs 24 hour); lowercase letters compare values in the same column (intergroup comparison: C+ vs C- vs PDT vs NaOCl+PDT vs PUI+PDT vs XP Endo+PDT). PDT: Photodynamic therapy, NaOCl: Sodium hypochlorite, PUI: Passive ultrasonic irrigation

Comparative analysis of bacterial counts (CFU/mL) at different times of final irrigation protocols combined with PDT compared to other groups revealed substantial reduction of bacterial populations. These were all significantly more effective compared to control groups; confirming the important role of irrigation in bacterial reduction. The determination of CFU inside the root canal for assessing the efficacy of disinfection methods has been applied in a number of *in vitro* studies [5,15]. Despite being low-cost and easy method for determining bacterial counts, it remains questionable whether CFU truly reflect bacterial growth inside the root canal.

In the present study, PDT without the use of sodium hypochlorite resulted in lower bacterial inhibition percentage (15.15% inhibition) compared to the other groups in which 2.5% NaOCl irrigant was used. It is evident that PDT combined with saline is insufficient to reduce microbial loads and the use of irrigation solutions with proven antimicrobial activity, such as NaOCl, is necessary. This fact corroborates Samiei *et al.* [6] suggesting that PDT may be an effective adjuvant method in root canal disinfection but cannot be used alone. Some studies have found that PDT significantly reduces *E. faecalis* counts in infected root canals when compared to traditional instrumentation/endodontic irrigation protocols [6,14, 26], but contradicting results have also been reported [9, 13, 27].

Similar to the present study, methylene blue as the photosensitizer and red light with 665 nm wavelength were used in other studies investigating *E. faecalis* elimination in root canals of extracted teeth; they reported bacterial reduction up to 97% [28, 29]. The results of these studies indicated the potential of PDT as an adjuvant antimicrobial procedure after chemo-mechanical preparation, but also highlighted the importance of an adequate or com PDT protocol for bacterial reduction in root canals.

Bumb *et al.* [30] used diode laser at 910 nm and power of 1 W, with pre-irradiation period of 10 min and three irradiation cycles for 20 sec, and observed higher efficacy in the PDT group compared to the control group submitted to conventional chemo-mechanical preparation. Ng *et al.* [31] evaluated the efficacy of PDT with red light (665 nm) using methylene blue as photosensitizer (concentration of 50 mg/mL), power of 1 W, and total energy dose of 30 J/cm^2. The pre-irradiation time was 2 min, followed by two irradiation cycles for 5 min with 2.5 min intervals. Teeth were divided into two groups: conventional cleaning of root canals (CC) and PDT+CC. The results showed the superior efficacy of PDT+CC compared to CC alone, in agreement with the present findings.

The studies of Soukos *et al.* [28] and Nagayoshi *et al.* [32]

showed that PDT is more effective in eliminating *E. faecalis* than conventional instrumentation/irrigation. However, these studies differ in terms of laser parameters used, including diode laser (635 nm, 805 nm and 628 nm wavelengths, respectively), output power (1 W, 1 W and 5 W, respectively), and photosensitizer type (methylene blue, indocyanine green and toluidine blue, respectively). In the studies by Bago *et al.* [14] and Rios *et al.* [33], the irradiation time was 60 and 30 sec, respectively. However, Pagonis *et al.* [2] and Foschi *et al.* [29] irradiated root canals for 5 and 10 min, respectively. Still, there is no consensus in literature regarding the most effective PDT parameters for *E. faecalis* elimination in dental root canals.

Some studies have reported superior antibacterial activity of PUI over conventional irrigation [34, 35] while others found no difference [36, 37]. In this study, antimicrobial activity was higher for PUI+PDT group compared to group submitted to conventional needle irrigation (C+). In the study by Ramazani *et al.* [8] , in the PUI group, the removal of residues from the apical third of the root was more effectively performed. According to Bao *et al.* [10], the different findings can be attributed to differences in the methodological designs, root canal anatomy, type of incubated bacteria/biofilm, instrumentation protocol, irrigation solution, PUI protocol, and conventional needle irrigation parameters. It may also be possible that areas not infiltrated by the irrigation were not sampled for microscopic analysis.

The present results are similar to those reported by Azim *et al.* [11] regarding bacterial reduction inside the root canal, with XP Endo providing significantly higher bacterial reduction (98.2%) than other irrigation techniques. Likewise, Bao *et al.* [10] using XP Endo as final irrigation protocol observed greater biofilm reduction in areas of difficult access in the root canal system compared to conventional irrigation and PUI. In the above study, samples obtained from the root canal also revealed greater bacterial reduction for XP Endo groups, at a rate of approximately 99%.

Enterococcus faecalis is able to penetrate dentinal tubules and to form biofilms, which represent a challenge for the dispersion of antibacterial agents [38]. Thus, microbiological sampling with absorbent paper points is a limitation of the present study, since it only allows analyzing the microbiota inside the main root canal. Consequently, bacteria present in areas inaccessible to mechanical debridement, such as small accessory canals or dentinal tubules, were not collected. Further studies are necessary to determine the efficiency of irrigation protocols in different canal anatomies and multi-radicular teeth. In addition, *in vivo* studies will provide the scientific basis so that the protocols tested can be safely used in clinical practice.

Conclusion

PDT combined with different final irrigation protocols was more effective in inhibiting *E. faecalis* than this therapy alone. In addition, XP Endo Finisher was the best irrigation protocol in inhibiting this microorganism.

Conflict of Interest: 'None declared'.

References

1. Siqueira JF, Rôças I. Clinical implications and microbiology of bacterial persistence after treatment procedures. J Endod. 2008;34(11):1291-301.
2. Pagonis TC, Chen J, Fontana CR, Devalapally H, Ruggiero K, Song X, Foschi F, Dunham J, Skobe Z, Yamazaki H, Kent R, Tanner AC, Amiji MM, Soukos NS. Nanoparticle-based endodontic antimicrobial photodynamic therapy. J Endod. (2010);36(2):322–8.
3. Ricucci D, Siqueira JF Jr. Fate of the tissue in lateral canal and apical ramifications in response to pathologic conditions and treatment procedures. J Endod. 2010;36(1):1-15.
4. Siddiqui SH, Awan KH, Javed F. Bactericidal efficacy of photodynamic therapy against Enterococcus faecalis in infected root canals: a systematic literature review. Photodiagnosis Photodyn Ther. 2013;10(4):632-43.
5. Afkhami F, Akbari S, Chiniforush N. Entrococcus faecalis elimination in root canals using silver nanoparticles, photodynamic therapy, diode laser, or laser-activated nanoparticles: An in vitro stud. J Endod. 2017;43(2):279-82.
6. Samiei M, Shahi S, Abdollahi AA, Eskandarinezhad M, Negahdari R, Pakseresht Z. The antibacterial efficacy of photo-activated disinfection, chlorhexidine and sodium hypochlorite in infected root canals: An in vitro study. Iran Endod J. 2016;11(3):179–83.
7. Mohammadi Z, Jafarzadeh H, Shalavi S, Palazzi F. Recent advances in root canal disinfection: A review. Iran Endod J. 2017;12(4):402–6.
8. Ramazani M, Asnaashari M, Ahmadi R, Zarenejad N, Rafie A, Yazadani Charati J. The effect of final rinse agitation with ultrasonic or 808 nm diode laser on coronal microleakage of root-canal treated teeth. Iran Endod J. 2018;13(1):108–13.
9. Marques da Silva B, Scaini F, Tomazinho FSF, Gonzaga CC, Leão Gabardo MC, Baratto-Filho F. Root preparation of deciduous teeth: Efficacy of waveone and protaper systems with and without passive ultrasonic irrigation. Iran Endod J. 2018;13(3):362–6.
10. Bao P, Shen Y, Lin J, Haapasalo M. In vitro efficacy of XP-endo Finisher with 2 different protocols on biofilm removal from apical root canals. J Endod. 2017;43(2):321-5.
11. Azim AA, Aksel H, Zhuang T, Mashtare T, Babu JP, Huang GT. Efficacy of 4 irrigation protocols in killing bacteria colonized in dentinal tubules examined by a novel confocal laser scanning microscope analysis. J Endod. 2016;42(6):928–34.
12. Amaral RR, Amorim JCF, Nunes E, Soares JA, Silveira FF. Photodynamic therapy in endodontics - review of literature. Rev. facul. odontol. 2010;15(2):207-11.

13. Souza LC, Brito PR, de Oliveira JC, Alves FR, Moreira EJ, Sampaio-Filho HR, Rôças IN, Siqueira JF Jr. Photodynamic therapy with two different photosensitizers as a supplement to instrumentation/irrigation procedures in promoting intracanal reduction of Enterococcus Faecalis. J Endod. 2010;36(2):292-6.
14. Bago I, Plečko V, Gabrić Pandurić D, Schauperl Z, Baraba A, Anić I. Antimicrobial efficacy of a high-power diode laser, photo-activated disinfection, conventional and sonic activated irrigation during root canal treatment. Int Endod J.2013;46(4):339-47.
15. Shen Y, Gao Y, Lin J, Ma J, Wang Z, Haapasalo M. Methods and models to study irrigation. Endod Topics. 2012;27(1):3-34.
16. Haapasalo M, Wang Z, Shen Y, Curtis A, Patel P, Khakpour M. Tissue dissolution by a novel multisonic ultracleaning system and sodium hypochlorite. J Endod. 2014;40(8):1178–81.
17. Nusstein JM. Sonic and ultrasonic irrigation. In: Bettina B, ed. Endodontic Irrigation: Chemical Disinfection of the Root Canal System. Switzerland: Springer. 2015:173–98.
18. FKG Swiss Endo. XP-endo Finisher: 3d generation. http://www.kkg.ch/sites/default/files/fkg_xp_endo_brochure_en_vb.pdf, 2016 (Accessed 13 August 2017).
19. Andrade FB, Arias MP, Maliza AG, Duarte MA, Graeff MS, Amoroso-Silva PA,Midena RZ, Moraes IG. A new improved protocol for in vitro intratubular dentinal bacterial contamination for antimicrobial endodontic tests: standardization and validation by confocal laser scanning microscopy. J Appl Oral Sci. 2015;23(6)591-8.
20. Christo JE, Zilm PS, Sullivan T, Cathro PR. Efficacy of low concentrations of sodium hypochlorite and low-powered Er,Cr:YSGG laser activated irrigation against an Enterococcus faecalis biofilm. Int Endod J. 2016;49(3):279-86.
21. Hannigan A, Lynch CD. Statistical methodology in oral and dental research: pitfalls and recommendations. J. Dent. 2013;41(5):385-92.
22. R. Larson, B. Farber. Estatística Aplicada. 6. ed. São Paulo: Pearson Prentice Hall, 2016.
23. Neelakantan P, Cheng CQ, Mohanraj R, Sriraman P, Subbarao C, Sharma S. Antibiofilm activity of three irrigation protocols activated by ultrasonic, diode laser or Er:YAG laser in vitro. Int Endod J. 2015;48(6):602–10.
24. Huang TY, Gulabivala K, Ng YL. A bio-molecular film ex-vivo model to evaluate the influence of canal dimensions and irrigation variables on the efficacy of irrigation. Int Endod J. 2008;41(1):60–71.
25. Falk KW, Sedgley CM. The influence of preparation size on the mechanical efficacy of root canal irrigation in vitro. J Endod. 2005;31(10):742–5.
26. Vaziri S, Kangarlou A, Shahbazi R, Nasab AN, Naseri M. Comparison of the bactericidal efficacy of photodynamic therapy, 2.5% sodium hypochlorite, and 2% chlorhexidine against Enterococcous faecalis in root canals: an in vitro study. Dent Res J. 2012; 9(5):613-8.
27. Hecker S, Hiller KA, Galler KM, Erb S, Mader T, Schmalz G. Establishment of an optimized ex vivo system for artificial root canal infection evaluated by use of sodium hypochlorite and the photodynamic therapy. Int Endod J. 2013;46(5):449-57.
28. Soukos NS, Chen PS, Morris JT, Ruggiero K, Abernethy AD, Som S, Foschi F, Doucette S, Bammann LL, Fontana CR, Doukas AG, Stashenko PP. Photodynamic therapy for endodontic disinfection. J Endod. 2006;32(10):979-84.
29. Foschi F, Fontana CR, Ruggiero K, Riahi R, Vera A, Doukas AG, Pagonis TC, Kent R, Stashenko PP, Soukos NS. Photodynamic inactivation of Enterococcus faecalis in dental root canals in vitro. Lasers Surg Med. 2007;39(10):782–7.
30. Bumb SS, Bhaskar DJ, Agali CR, Punia H, Gupta V, Singh V, Kadtane S, Chandra S. Assessment of photodynamic therapy (PDT) in disinfection of deeper dentinal tubules in a root canal system: An in vitro study. J Clin Diagn Res. 2014;8(11):67–71.
31. Ng R, Singh F, Papamanou DA, Song X, Patel C, Holewa C, Patel N, Klepac-Ceraj V, Fontana CR, Kent R, Pagonis TC, Stashenko PP, Soukos NS. Endodontic photodynamic therapy ex vivo. J Endod. 2011;37(2): 217-22.
32. Nagayoshi M, Nishihara T, Nakashima K, Iwaki S, Chen KK, Terashita M, Kitamura C. Bactericidal effects of diode laser irradiation on Enterococcus faecalis using periapical lesion defect model. ISRN Dentistry. 2011; 2011(1):1-6.
33. Rios A, HE J, Glickman GN, Spears R, Schneiderman ED, Honeyman AL. Evaluation of photodynamic therapy using a light-emitting diode lamp against Enterococcus faecalis in extracted human teeth. J Endod. 2011;37(6):856-9.
34. Harrison AJ, Chivatxaranukul P, Parashos P, Messer HH. The effect of ultrasonically activated irrigation on reduction of Enterococcus faecalis in experimentally infected root canals. Int Endod J. 2010;43(11):968–77.
35. Layton G, Wu WI, Selvaganapathy PR, Friedman S, Kishen. A Fluid dynamics and biofilm removal generated by syringe-delivered and 2 ultrasonic-assisted irrigation methods: A novel experimental approach. J Endod. 2015;41(6):884–9.
36. Bhuva B, Patel S, Wilson R, Niazi S, Beighton D, Mannocci F. The effectiveness of passive ultrasonic irrigation on intra-radicular Enterococcus faecalis biofilms in extracted single-rooted human teeth. Int Endod J. 2010;43(3):241–50.
37. Paiva SS, Siqueira JF Jr, Rôças IN, Carmo FL, Leite DC, Ferreira DC, Rachid CT, Rosado AS. Molecular microbiological evaluation of passive ultrasonic activation as a supplementary disinfecting step: a clinical study. J Endod. 2013;39(10):190-4.
38. Du T, Wang Z, Shen Y, Ma J, Cao Y, Haapasalo M. Effect of long-term exposure to endodontic disinfecting solutions on young and old Enterococcus faecalis biofilms in dentin canals. J Endod. 2014;40(4):509-14.

Please cite this paper as: de Vasconcelos Neves G, dos Santos KSA, de Souza Sales Rocha EAL, de Moura RQ, Morais Barros DG, Gominho LF, de Castro Gomes DQ. Antibacterial Effect of Photodynamic Therapy on Root Canal Disinfection Combined with Different Irrigation Protocols. Iran Endod J. 2020;15(2): 90-5. *Doi*: *10.22037/iej.v15i2.27801.*