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Research Article

Evaluation of Competency of Diode Laser and Er: YAG Laser Activation on Sodium Hypochlorite Capability for Biofilm Eradication and Smear Layer Removal: A Comparative *In vitro* Study

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Abstract

Objective: This study attempted to evaluate the efficacy of Sodium hypochlorite activation using two types of laser; Diode laser and Er: YAG laser utilizing PIPS tip as compared to conventional Sodium hypochlorite syringe irrigation on biofilm eradication, smear layer removal and topographic surface changes.

Methods: For biofilm eradication analysis, 21 single-rooted premolar human teeth were prepared and inoculated with E. faecalis, then divided into three groups of seven roots each. All teeth were subjected to irrigation with 10 ml 2.5% Sodium hypochlorite either with, conventional syringe irrigation (CSI) in Group I, Diode laser activation (940 nm) in Group II, or Er: YAG laser activation utilizing PIPS tip in Group III. Biofilm eradication was evaluated using Confocal Laser Scanning Microscope CLSM. For comparing the smear removal efficacy and surface topographic changes, another 21 single-rooted premolar human teeth, were irrigated with saline during instrumentation and assigned to three groups as mentioned. Scanning Electron microscope was used to score the presence of a smear layer at different root canal levels and analyze the topographic surface changes by detecting the presence of dentinal tubules changes.

Results: Er: YAG Group utilizing PIPS tip showed significantly higher biofilm eradication when compared to CSI Group and Diode laser activation Group ($p < 0.0001$ and 0.004 respectively). SEM analysis presented Group III with significantly higher smear layer removal in the coronal and middle thirds as compared to the other two Groups ($p = 0.031$). Analysis of topographic surface changes showed a statistically significant higher incidence of dentinal tubules changes in both laser activation groups when compared to CSI only in the coronal third ($p = 0.04$).

Conclusions: Activation of Sodium hypochlorite irrigant using Er: YAG laser utilizing PIPS technique enhanced the biofilm eradication capability and smear layer elimination potentiality. Yet, the dentinal tubules changes remain higher when laser is used.

Keywords: LAI Laser Activated Irrigation; PIPS Photon Initiated Photoacoustic Streaming; Biofilm Smear Layer; Dentin Changes; Irrigation; Confocal Laser Microscopy; Scanning Electron Microscope

Introduction

The main goal of endodontic treatment is to achieve efficient disinfection of the root canal system, and to prevent its recontamination. The complex anatomy of the root canal system often prevents the irrigant penetration into recesses that cannot be reached by mechanical instrumentation [1]. Therefore, to enhance the efficiency of the irrigating solutions, various agitation techniques either manual or machine- assisted had been offered, including laser devices [2].

The use of Diode laser with irrigant enhances the irrigant efficacy by increasing its temperature, and by inducing cavitations in its water-based media. Despite the emergence of other types of laser for root canal disinfection, Diode laser remains the most preferred choice due to its favorable antibacterial properties, relatively safe wavelength, minimal temperature rise, and cost effectiveness [3].

On the other hand, Er: YAG laser has a higher wavelength than Diode laser (2940 nm) with the highest absorption in water, which makes it suitable to be used in root canal treatment. When laser pulses are focused into a limited volume of fluid, plasma is generated. This causes rapid heating of the material, forming cavitations, followed by an explosive expansion and emission of a shock wave that results in secondary microcavitations. This technique is referred to as Photon-initiated photoacoustic streaming (PIPS) [1].

Up till now, the data regarding the efficacy of Diode laser and Er:YAG laser on activation of Sodium hypochlorite irrigant is still lacking. Therefore, this study was undertaken to evaluate the efficacy of Sodium hypochlorite activation in human extracted single rooted teeth using two types of laser; Diode laser and Er:YAG laser using PIPS tip as compared to conventional Sodium hypochlorite needle irrigation on biofilm eradication, smear layer removal and topographic surface changes of dentin. The null hypothesis was that there would be no significant difference between Sodium hypochlorite activation using Diode laser, Er:YAG laser utilizing PIPS tip and conventional Sodium hypochlorite needle irrigation on biofilm eradication, smear layer removal and topographic surface changes of dentin.

Materials and Methods

sample size

Based on the previous study by Zhu., *et al*. 2013 [4] and using power 80% and 5% significance level, sample size was calculated as 42 samples for the total study, utilized for 2 outcomes analysis. Thus, 21 samples were utilized for each outcome. The samples of each outcome were distributed among 3 experimental groups; each containing 7 samples per outcome.

Part(I): Evaluation of biofilm eradication

Root canal preparation, inoculation and disinfection

Twenty-one permanent single-rooted premolar human teeth were utilized in this study. The teeth were extracted for periodontal disease or orthodontic treatment were collected from the Department of Oral and Maxillofacial surgery at the Faculty of Dentistry-Cairo University. External root surfaces of teeth were cleaned with a curette to remove calculus and periodontal tissues, and then placed in Sodium hypochlorite (NaOCl) (Clorox Co., Oakland, CA, USA) for 30 min to remove soft tissue debris. Afterward, teeth were stored in saline till use. Conventional radiograph was used to confirm that each tooth had a single canal with no internal calcifications, irregularities or any other anomalies. Crowns were flattened using a low speed diamond saw under copious irrigation to obtain approximately 15 mm uniform root lengths. Patency of the canals was established using k file #10 (MANI, Matsutain Seisakusho Co., Tochigi- Ken, Japan), then root canals were instrumented using Protaper Next rotary system (Dentsply Sirona, York, Pennsylvania, USA) starting with X1 (#17/4), X2 (#25/6), X3 (#30/7) and finally X4 (#40/6) using X-Smart Endo Motor (Dentsply Sirona, York, Pennsylvania, USA) with speed of 300 RPM and 2.5 N.Cm torque for all the files. Copious irrigation with 10 ml 2.5% NaOCl was used throughout the root canal instrumentation, 2.5 ml of NaOCl were used between each successive instrument and as final flush with a rate of 1ml/min. Irrigation was done using disposable plastic syringe with 30G end-vented needle (Sung Shim Medical Co., Bucheon, Gyeonggi, South Korea) reaching 2 mm short of the total working length using a total volume of 10 ml NaOCl per canal. The root canal was then irrigated with 3 ml saline (FIPCO, Borg Elarab, Alexandria, Egypt), followed by 3 ml of 17% Ethylenediaminetetraacetic acid (EDTA) (Prevest Dentpro, Ltd., Jammu, India) to remove the smear layer. Finally, 3 ml of sterile saline (FIPCO, Borg Elarab, Alexandria, Egypt) was used as a final flush. Sealing of the apical foramen was performed by applying a Filtek TM supreme Ultra Flowable composite (3M ESPE, St Paul MN, USA) on the root apex. Teeth were sterilized by autoclaving for 15 min at 121°C using Hygenius autoclave (FONA Dental s.r.o., Bratislava, Slovak Republic). Twenty microns of E. faecalis (ATCC 29212) suspension was used to infect the root canals using a micropipette (Thermo Fisher Scientific, Waltham, Massachusetts, USA) and then teeth were immersed in brain heart infusion broth media (BHI) (Sigma-Aldrich, St. Louis, Missouri, USA). The flasks with infected teeth were incubated aerobically using HerathermTM Advanced Protocol Microbiological Incubator (Thermo Fisher Scientific, Waltham, Massachusetts, USA) for 3 weeks at 37°C with gentle daily shaking.

Experimental groups distribution

- Group (I): (n = 7) Control group, conventional syringe irrigation with 10 ml 2.5% NaOCl (Clorox Co., Oakland, CA, USA) using disposable plastic syringe with 30G open-ended needle (Sung Shim Medical Co., Bucheon, Gyeonggi, South Korea) reaching 2 mm short of the total working length, with a rate of 1ml/min.
- Group (II): (n = 7) 2.5% NaOCl activated with Diode laser, the root canals were irrigated with a total volume of 10 ml NaOCl. The irrigation/activation protocol was as follows: 2 ml 2.5% NaOCl for 5 seconds time periods, then Diode laser activation for another 5 seconds. This cycle was repeated for 4 times, then final irrigation with 2 ml 2.5% NaOCl was done with no activation. The total volume of NaOCl used was 10 ml and the total laser activation time was 20 seconds. For the activation process, Biolase Epic XTM (Biolase, Irvine, California, USA) was utilized using E2-14 tip (Biolase, Irvine, California, USA); endo 200-µm flexible laser tip with 14 mm length, at 1 mm short of working length, at a wave length of 940nm ± 10nm with standardized settings of 2 watt. The laser tip was being removed in slow, helical movements and in an apico-coronal direction to ensure that each part of the canal was irradiated (Figure 1) [5].
- Group (III): (n=7) 2.5% NaOCl activated with PIPS tip using Er:YAG laser with a wavelength of 2940 nm. LightWalker® by Fotona (Fotona d.o.o., Ljubljana, Slovenia) was used with a quartz cylindrically tapered PIPS® tip 400/14 (Fotona d.o.o., Ljubljana, Slovenia) figure 2. The laser operating parameters were 20 mJ per pulse, 15 Hz, and 50 µs pulse duration. Both the air and water spray features of the laser unit were set to ''off''. Artificial pulp chamber was made in teeth where crown was almost removed during the flattening process; to mimic the coronal structure with a composite buildup of 3 mm length using Filtek™ Z350 XT (3M ESPE, St Paul MN, USA), then the canal and pulp chamber were bathed in 2.5% NaOCl. The PIPS tip was placed into the coronal access opening of the pulp chamber only, and kept stationary without being advanced apically into the root canal during activation process. The laser activation protocol was as follows: 30 seconds on, then 30 seconds off, and this cycle was performed three times (i.e., total of 90 sec of activation). The amount of NaOCl solution used during each 30 sec exposure was 4 ml, 3 ml and 3 ml respectively, hence the total NaOCl irrigation volume used was 10 mL [6].

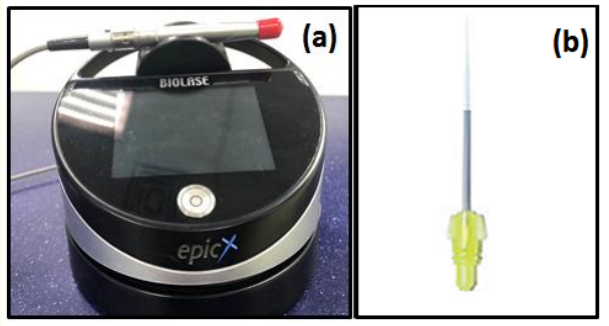

Figure 1: Showing the Biolase Epic XTM diode laser device (a), and E2-14 endo tip 200-µm flexible laser tip with 14 mm length (b).

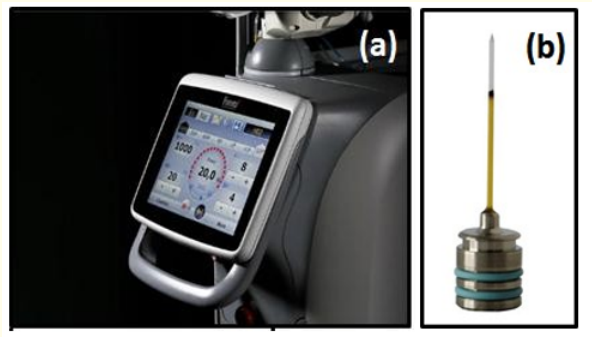

Figure 2: Showing LightWalker® Er:YAG laser machine by Fotona (a), and PIPS® 400/14 a cylindrical, conically tapered, 14 mm long 400 µm diameter quartz tip (b).

Evaluation of biofilm eradication by confocal laser scanning microscope (clsm)

The disinfected teeth from each group were sectioned into 4x2 mm size specimen with 1 mm thickness using IsoMetTM precision sectioning saw (Buehler Ltd., Lake Bluff, IL, USA). Apical root section from each group was stained with fluorescent LIVE/DEAD BacLight Bacterial Viability stain (Sigma-Aldrich, St. Louis, Missouri, USA) then was viewed using a confocal laser scanning microscope (Carl Zeiss, Göttingen, Germany). Simultaneous dual-channel imaging was used to display the green fluorescence (live cells) and red fluorescence (dead cells). Quantification of the CLSM images was done at two to three random areas using ZEN 2012 (blue edition) software (Carl Zeiss, Göttingen, Germany). The fluorescence from

the live and dead bacterial cell was calculated by measuring the fluorescent light intensity. The volume ratio of red fluorescence to green-and-red fluorescence in the images indicated the portion of dead cells out of the total cells. The following equation was used:

$$percent = \frac{intensity\ \ of\ \ red}{intensity\ \ of\ \ red + intensity\ \ of\ \ green} \times 100$$

Part (II): evaluation of smear layer removal and surface topographic changes

Another 21 single-rooted human teeth were recruited and similarly prepared as previously mentioned, except that sterile saline irrigation was used throughout instrumentation. Experimental group distribution and root canal management were conducted as before. After different irrigation/irradiation procedures, the roots were split longitudinally for SEM observation. The coronal, middle, and apical thirds of the root canal were examined individually in each specimen. Each specimen was coated with gold stain using EMITECH K550X sputter coater (Emitech Ltd, Ashford, UK). Then, the coronal, middle, and apical thirds of the root canal were examined individually using QuantaTM 250 FEG SEM (FEI company, Hillsboro, Oregon,USA), with magnification of 2000X and 5000X for smear layer evaluation.(4) The SEM photographs were evaluated by two blinded observers using Takeda., *et al*. scoring method for evaluating smear layer:(7)

- Score 1: No smear layer and debris evidence on dentinal tubules.
- Score 2: A few regions of dentinal tubules covered with a smear layer and debris.
- Score 3: Most regions of dentinal tubules covered with smear layer and debris, a few tubules cleaned and opened.
- Score 4: Dentinal tubules completely covered with smear layer and debris.

Samples were examined at 500X for evaluation of topographic surface changes [8] Photomicrographs were used for detecting the presence of dentinal tubules changes.

The statistical analysis

Data was analyzed using IBM SPSS advanced statistics (Statistical Package for Social Sciences), version 21 (SPSS Inc., Chicago, IL). Numerical data was described as mean and standard deviation or median and range. Data was explored for normality using Kolmogrov-Smirnov test and Shapiro-Wilk test. Comparisons of the 3 groups for normally distributed numeric variables was done using the ANOVA while for non normally distributed numeric variables were done by Kruskal Wallis test. A p-value less than or equal to 0.05 was considered statistically significant. All tests were two tailed.

Results

The results of biofilm eradication showed a statistically significant difference between the three groups when applying ANOVA test ($p < 0.0001$) (Table 1). On the other hand, Tukey post hoc test for pairwise comparison of the three groups revealed no statistically significant difference between Group I (CSI) and Group II (diode laser activation) ($p = 0.065$), with a statistically significant difference between Group I (CSI) and Group III (Er:YAG laser activation utilizing PIPS tip) ($p < 0.0001$), and between Group II (diode laser activation) and Group III (Er:YAG laser activation utilizing PIPS tip) ($p = 0.004$) (Table 2, Figure 3).

	Group I	Group II	Group III	P – Value
Mean	39.3%	45.5%	55.3%	<0.0001
SD	3.1%	6.5%	4.2%	

Table 1: Mean, SD values and results of ANOVA test for comparison of biofilm eradication percentages among the three groups.

P – Value		
Group I	Group II	0.065
	Group III	<0.0001
Group II	Group III	0.004

Table 2: Results of Tukey post hoc test for pairwise comparison of biofilm eradication percentage among the threegroups.

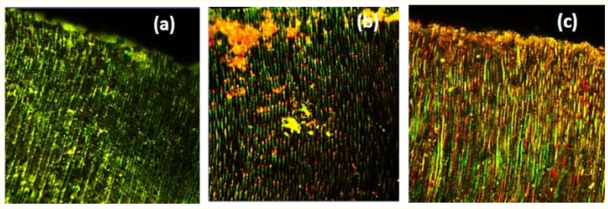

Figure 3: CLSM images representing biofilm eradication in conventional syringe irrigation (CSI) Group (a), Diode laser activation Group (b), Er:YAG laser activation utilizing PIPS tip Group (c).

Regarding the smear layer removal, Chi square test presented a statistically significant difference in the scores of smear layer removal in Group III as compared to the other two Groups in the coronal and middle thirds (p = 0.031). However, there was no statistically significant difference in the scores of smear layer removal between the three groups in the apical third (p = 0.44) (Table 3, Figure 4).

Coronal third score	Group I		Group II		Group III		
	N	%	N	%	N	%	P-Value
	0	0.0%	0	0.0%	4	57.1%	0.031*
	5	71.4%	5	71.4%	3	42.9%	
	2	28.6%	2	28.6%	0	0.0%	
	0	0.0%	0	0.0%	0	0.0%	
Middle third score	**Group I**		**Group II**		**Group III**		
	N	%	N	%	N	%	P - Value
	0	0.0%	0	0.0%	3	42.9%	0.016*
	3	42.9%	0	0.0%	3	42.9%	
	4	57.1%	5	71.4%	1	14.2%	
	0	0.0%	2	28.6%	0	0.0%	
Apical third score	**Group I**		**Group II**		**Group III**		
	N	%	N	%	N	%	P - Value
	0	0.0%	0	0.0%	0	0.0%	0.440
	0	0.0%	1	14.3%	2	28.6%	
	3	42.9%	1	14.3%	1	14.3%	
	4	57.1%	5	71.4%	4	57.1%	

Table 3: Frequencies (N), Percentages (%) and results of Chi square test for comparison of smear layer removal scores in the coronal, middle and apical thirds between the three groups*significant at p<0.05.

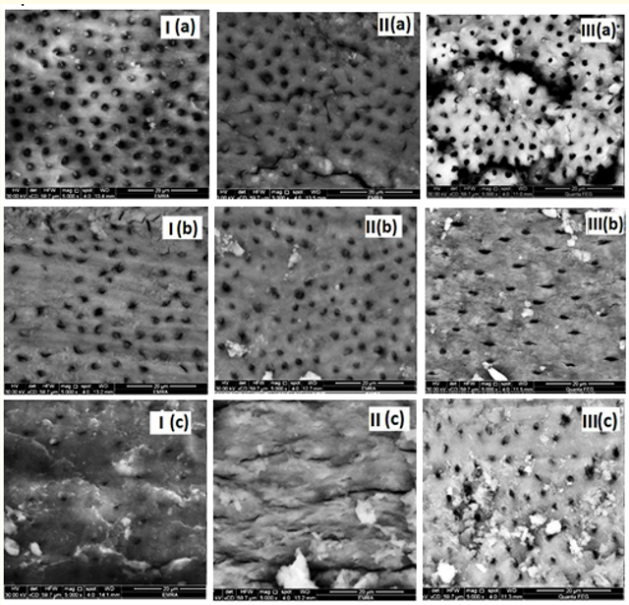

Figure 4: SEM images representing smear layer removal in conventional syringe irrigation (CSI) Group (I), Diode laser activation Group (II) Er:YAG laser activation utilizing PIPS tip Group (III), in coronal third (a), middle third (b), and apical third (c), each at magnification 5000X.

Regarding topographic surface changes, Chi square test presented higher dentinal tubules changes in both laser activation groups when compared to CSI only in the coronal third with a statistically significant difference between CSI and the other two groups (p = 0.04) (Table 4, Figure 5).

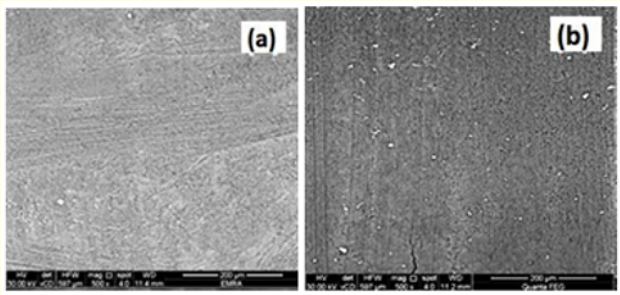

Figure 5: SEM images representing dentinal tubules changes in Diode laser activation Group (a) and Er:YAG laser activation utilizing PIPS tip Group (b) in the coronal third (c), at magnification 500X.

Coronal third	Group		Group II		Group III		
	N	%	N	%	N	%	P-Value
	7	100%	3	42.90%	3	42.90%	0.04*
	0	0%	4	57.10%	4	57.10%	
Middle third	**Group I**		**Group II**		**Group III**		
	N	**%**	**N**	**%**	**N**	**%**	**P-Value**
	7	100%	3	42.90%	5	71.40%	0.061
	0	0%	4	57.10%	2	28.60%	
Apical third	**Group I**		**Group II**		**Group III**		
	N	**%**	**N**	**%**	**N**	**%**	**P-Value**
	7	100%	5	71.40%	4	57.10%	0.077
	0	0%	2	28.60%	3	42.90%	

Table 4: Frequencies (N), Percentages (%) and results of Chi square test for comparison of the presence of dentinal tubules changes in the coronal, middle and apical thirds between the three groups: *significant at p<0.05.

Discussion

Single rooted teeth with single canal were selected for this study; since the oval cross section of the single canal can't be ultimately touched and cleaned by the rounded cross sectional design of most endodontic files, leaving large areas of untouched canal walls and accumulated hard-tissue debris in irregularities within the root canal space [9-12]. Therefore, the role of irrigant activation in such canals is optimized as the elimination of bacteria located in these areas totally depends on the efficacy of irrigating solution.

The *E. faecalis* was used to infect the root canals; since *E. faecalis* was found to be the most common and occasionally the only single isolated bacteria from root canals of teeth with persistent periapical periodontitis [13-17]. *E. faecalis* (ATCC 29212) strain was selected as it has the ability to grow in a biofilm state with enhanced adhering capacity, increased virulence factors and higher resistance to antimicrobial agents [18-22].

Testing biofilm eradication in the apical region was carried out as this region is often precluded from contact or disinfection by the irrigant due to the vapor lock phenomenon [20]. Moreover, studies concluded that the root apical third harbors the highest percentage of microorganisms with the presence of canal ramifications, accessory canals and lateral branches, rendering it a microbial threat for the ideal root canal disinfection procedure [21].

For irrigant activation using Diode laser, the optical fiber was placed inside the root canal 1mm shorter than the working length and removed in slow, helical movements and in an apico-coronal direction to overcome the problem of parallel emission of laser energy from the tip of the optical fiber and to ensure that each part of the canal was irradiated obtaining uniform coverage of the canal surface [22].

For irrigant activation using Er:YAG laser, PIPS tip was used and was only held in the pulp chamber, in contrast to other tips which need to be inserted inside the root canal. Such need was replaced by the photoacoustic shockwaves induced with the pulsed laser in the irrigant travelling throughout the root canal system, allowing its 3-D movement without the need for intracanal tip insertion.

The significant difference in biofilm eradication between the Er:YAG laser group utilizing PIPS tip and conventional syringe irrigation group as well as between Er:YAG group and Diode laser group, might be attributed to the fact that in mid-Infrared lasers, such as Er:YAG laser, the target chromophore is the water molecule [23]. Er:YAG laser interacts with the water present in the aqueous medium of irrigant producing primary and secondary cavitation effects, which is described as the formation of vapor- containing bubbles inside a fluid [24]. In the root canal environment, such shockwaves could potentially disrupt bacterial biofilms, rupture bacterial cell walls, and remove smear layer and debris. On the

contrary, Diode laser interacts with tissues by diffusion resulting in a photothermal effect [25]. Near infrared lasers, including Diode laser, show lack of affinity to water. Thereby, irrigant activation by Diode laser occurs through heating of fluids rather than agitation or cavitation. These results are in accordance with what was concluded before by Peters., *et al*. (2011) [26], Cheng., *et al*. (2012) [27], Ordinola-Zapata., *et al*. (2013) [1], Al Shahrani., *et al*. (2014) [28], Mathew., *et al*. (2014) [29], Cheng., *et al*. (2017) [30] and Golob., *et al*. (2017) [31] who demonstrated that Er: YAG Laser activation of Sodium hypochlorite using PIPS technique significantly improved the *E. faecalis* biofilm eradication.

Regarding the smear layer removal, the significant difference between the Er:YAG group utilizing PIPS tip and the other two groups might be attributed to the higher potentiality of mid-infrared erbium lasers to be absorbed by water, rendering them more efficient in smear layer removal [7,32]. Moreover, impulsive activation of irrigant at every laser pulse results in shockwave generation. Mid-infrared laser systems with short pulse durations can induce pressure waves in water [33]. These laser-generated pressure waves move at high speed, with different characteristics which proved to enhance the action of endodontic irrigants in terms of smear layer removal [34]. The limited smear layer removal at the apical third could be explained by the fact that the coronal dentin is exposed to a higher volume of irrigant with better flow when compared to the apical dentin; as the diameter of the root canal decreases on moving from coronal to apical third. Moreover, the stationary placement of the laser tip at the coronal third, might also result in a less effective irrigant activation at apical third of canal. Unfortunately, it is still unknown as to what extent the rapid flow and the action of cavitation bubbles can contribute to root canal cleaning [35]. These results are in accordance with what was stated by Zhu., *et al*. (2013) [4]. who concluded that PIPS-aided irrigation of NaOCl can significantly remove smear layer in the coronal and middle thirds of single-rooted teeth, but cannot effectively remove the smear layer in the apical third of the root canal.

The significantly higher frequency of dentinal tubules changes in Diode laser activation group and Er:YAG group utilizing PIPS tip might be attributed to the fact that each type of laser interacts differently with the hard dental tissues [36-38]. Diode laser is not well absorbed in water nor in the mineral matrix. Therefore, scattering of Diode laser is more predominant than absorption in aqueous media [39]. As a result, deeper penetration in the tissue is expected and rather thermal effects are observed. When diode laser is utilized for irrigant activation, part of the energy is absorbed by the mineral structures of dentin as phosphate and carbonate, disarranging their crystalline arrangement [40]. This structural transformation appears morphologically as melting of intracanal dentin and partial to complete obliteration of dentinal tubules. These results were in accordance with the results reported by Alfredo., *et al*. (2009) [41]. and Saghiri., *et al*. (2012) [42]. who proposed that Diode laser caused fusion and re-solidification of dentin and thus reduce dentin permeability.

Similar effect was observed when using Er:YAG laser. This could be attributed to its wavelength (2940 nm) which shows increased absorption in water and hydroxyapatite crystals. As a result of its high affinity to (OH) ion in water molecules, this wavelength ablates intertubular dentin more than peritubular dentin due to its lower mineral content and higher water-containing organic matrix, causing dentinal tubules changes [43,44].

Conclusions

Within the limitations of this study, it could be concluded that none of the root canal disinfection methods resulted in complete *E. faecalis* biofilm eradication. However, Sodium hypochlorite irrigant activation using Er:YAG laser and PIPS technique augmented its biofilm eradication capability, especially in the apical third and enhanced the smear layer elimination. Moreover, the combination of Sodium hypochlorite and laser minimized the craters and microcracks formation. Yet, the dentinal tubules changes remain higher when laser is used.

Conflict of Interest

The authors deny any conflicts of interest in this study.

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