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Antibacterial Efficacy of Photoactivated Irrigation of Diode and Photon-Induced Photoacoustic Streaming (PIPS) Using Er-Yag Lasers on Infected Teeth

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ABSTRACT

Background: *Enterococcus faecalis* is a robust microorganism possessing a diverse array of virulence elements, including lytic enzymes, aggregation substance and lipoteichoic acid. It can also withstand harsh conditions, resist intracanal medicaments, and survive the hard conditions present in root-canal treated teeth. **Aim of the work:** To assess the impact of 980 nm diode laser photo-activated irrigation and photon-induced photoacoustic streaming (PIPS) - 2940 nanometer Erbium-YAG laser on antibacterial efficacy against the biofilm of Enterococcus faecalis. **Methods:** Forty-five, single canaled teeth were used. The teeth were evenly distributed among three experimental groups according the final irrigation protocol to: Group I (lateral-vented needle) (n=15), Group II (Diode laser 980nm) (n=15) (Continuous mode with power 2W) and Group III (Er-YAG laser 2940nm) (n=15) (Pulsed SSP mode, 20mJ, 15Hz and 0.3W). The quantity of irrigant was 4ml NaOCl and activated for 40 seconds in different groups. Each group was split evenly into two subgroups according to the evaluation method: (A) Colony Forming Units (n = 7) and (B) Confocal Laser Scanning Microscope (n = 7). **Results:** CFUs results showed that both diode and Er:YAG lasers significantly reduced bacterial counts (97.87%) and (99.79%) respectively than lateral vented needle group (95.21%) with statistically significant difference. Additionally, a significant statistical difference was observed between the Er:YAG and diode laser irrigation activation groups. **Conclusion:** CLSM results demonstrated a significant statistical variation in CLSM showed in bacterial eradication between groups where it was found in (Er-YAG) (93.75%) followed by (Diode) (77.05%) and (Side vented needle) (10.79%). The best results for laser activation were observed with PIPS, which resulted in superior disinfection with a higher bacterial reduction. The diode laser group demonstrated the next highest level of bacterial reduction. In contrast, the lateral-vented needle group demonstrated the lowest level of bacterial reduction among all groups.

Keywords: Diode laser, Er-YAG laser, Enterococcus faecalis, activated irrigant solutions.

1. Introduction

When microorganisms infiltrate and colonize the dental pulp, root canals infections occur. As this microbial colonization producing metabolic by-products quickly reach the perirapical area through the apical foramen or lateral canals, they induce a cascade of inflammatory responses. Conventional procedures of endodontic treatment using mechanical tools and disinfection agents showed difficulty in reaching these microorganisms, over than one-third of the root canal may still remain infected, with microorganisms persisting within the intricate network of the tubules, which cannot be accessed by

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root canal cleansing agents (Mandras *et al*., 2020; Cheung *et al.,* 2019; Golg *et al.,* and Linh *et al*., 2020).

Additionally, intra-canal medications have a restricted antibacterial action and a little diffusion ability into the dentinal tubules. These disadvantages can contribute to the lack of success of RCT and the development of infection periapically due to the apical influx of bacteria (Mathew *et al.,* 2014; Meire *et al.,* 2012; Bago *et al.,* 2013).

Enterococcus faecalis is a robust microorganism possessing a diverse array of virulence elements, including lytic enzymes, aggregation substance, and lipoteichoic acid. This organism can be disseminated within the intricate network of dentinal tubules for prolonged periods, maintaining their viability, adhering to and forming biofilms on dentin. E. faecalis can also withstand harsh conditions, resist intracanal medicaments, and survive the hard conditions present in root-canal treated teeth. Which made the effective biofilm removal is crucial for root canal disinfection. For these reasons, proper irrigation and high cleaning are crucial to enhance sealer efficacy, facilitating its extension within the dentinal tubules (Wu *et al.,* 2014; Dioguardi *et al*., 2018) [8, 9]. Traditionally, root canal cleaning rely on the use of disinfecting agents (Chemical phase/ Irrigating solutions) and mechanical instrumentation (Mechanical phase/ Irrigating protocols). The commonly used irrigants include sodium hypochlorite (NaOCl), chlorhexidine, ethylenediaminetetraacetic acid, and citric acid (Dioguardi *et al*., 2018). Sodium hypochlorite continues to be the standard root canal irrigant due to its well-established broad-spectrum antimicrobial properties and its ability to effectively dissolve necrotic tissue and organic matter (Pintor *et al*., 2016) [10]. However, the antimicrobial action of NaOCl can be compromised by the exudates at the periapical area. Additionally, NaOCl does not consistently eliminate E. *faecalis* biofilms from the root canals (Jena *et al*., 2015; Rath *et al.,* 2020; Li *et al.,* 2020; Tosić *et al.,* 2016; Pradeep *et al.,* 2020; Alghamdi *et al.,* 2020 and Wang *et al*., 2012).

Endodontic irrigation methods can be categorized into two broad types: hand and automated irrigation methods. Hand irrigation often involves the use of a plastic syringe to deliver irrigant solution under positive pressure. Automated irrigation encompasses sonic and ultrasonic methods. Furthermore, more advanced devices like the apical negative pressure irrigation, vibratory files, and lasers (Lukac *et al*., 2017; Godiny *et al.,* 2020 and Olivi, 2013). Researches had demonstrated that effective irrigation is limited to needle tip area. Thus, it is suggested for the placement of the needle near the apex to reduce NaOCl extrusion. While NaOCl has antimicrobial properties, it possesses decomposing characteristics which cause significant tissue irritation. Additionally, applying excessive pressure with a traditional needle during irrigation may lead that the irrigant extrude to the apical tissues (Lukac *et al*., 2017). To overcome problems and risks faced during traditional endodontic needle irrigation; various activation techniques have established aiming to enhance the irrigant solutions efficiency (Lukac *et al*., 2017).

Laser techniques in endodontic treatments include; Direct laser irradiation (DLI) where no irrigant used and the tip emitting laser is placed into the canal producing photothermal effect. Laser-activated irrigation involves placing the laser tip inside the canal while the irrigant is present, utilizing the photothermal effect. In contrast, photon-induced photoacoustic streaming involves positioning its tip at the upper part of the canal, the presence of the irrigant is a must, to leverage the photoacoustic effect (Olivi, 2013; Korkut et al., 2018; Suer et al., 2020 and Al Shahrani *et al*., 2014).

Using Diode laser inside the root canal showed promising both bactericidal and cleaning effects as well as closing dentinal tubules (Al-Mafrachi *et al*., 2018; Kaiwar *et al*., 2013).
The enhanced efficacy of diode lasers can be attributed to their superior penetration depth, which can reach up to 1000 µm into the dentinal tubules, significantly surpassing 100 µm flow depth of chemical disinfectants (Preethee *et al*., 2012).

However, it should be noted that there is a limited affinity between diode wavelengths (near-infrared lasers at 940 nm and 980 nm) and water. As a result, higher power settings are necessary to generate sufficient heating of fluids, where it induces cavitation within the lumen of the root canal. This process may enhance smear layer and debris removal, resulting in improved bactericidal effect (Preethee *et al*., 2012; Olivi *et al.,* 2014 and Matsumoto *et al*., 2011). Also, PIPS protocol was used which depends on photo acoustic effect, it introduced improvements to the available utilized laser-assisted techniques that involve stronger laser energy and prolonged pulses durations (De Moor *et al*., 2010; Lloyd *et al.,* 2014 and DiVito *et al*., 2012).

Photon-induced photoacoustic streaming (PIPS) employs a 2940 nm Erbium: YAG laser equipped

with a specialized tip to deliver ultralow energy levels, thereby transferring energy into the irrigation solutions while maintaining a negligible elevation of tooth temperature. PIPS activated irrigation demonstrated the generation of powerful and fast shockwaves, which can effectively improves irrigant flow and microbial removal resulting with efficient cleaning (DiVito *et al*., 2012).

Earlier studies have indicated that 2940 nm wavelength of the Erbium :YAG laser to be employed with the PIPS system since it is highly absorbed in water. The sub-ablative pulsed energy of 20 mJ at 15 Hz is used to achieve efficient activation of solutions inside root canals, at the same time minimizing the temperature elevation of laser radiation on the canal walls (George and Walish, 2010; Dewsnup *et al*., 2010; Gregorio *et al.,* 2010; Baz *et al.,* 2012; Attiguppe *et al.,* 2018 and Wang *et al*., 2018). Its antibacterial impact is attributed to its affinity to OH groups and water. Resulting in the destruction of E. *faecalis* upon the absorption of energy into the bacteria (Wang *et al*., 2018; Dai *et al*., 2018 and Mathew *et al*., 2014).

Many methods can be used to evaluate antibacterial activity including the agar diffusion test, direct contact test and by measuring the kinetics of bacterial growth. Also, CFUs evaluation methodology's level of sensitivity is one of the most commonmethods used but it is postulated that this method may be inadequate in the detection of viable cells at lower concentrations. CLSM analysis is a suitable method for locating and quantifying bacteria within dentinal tubules. This technique offers valuable insights into the degree of dentin infection and the viability of the bacterial population, aiding in the assessment of treatment efficacy and prognosis. It holds promise for clarifying the consequence of remaining bacteria in an uncultivated condition (Matos *et al*., 2012; Giovanni, 2013; Olivi et al., 2021; Thomas *et al.,* 2017, Mehrvarzfar *et al.,* 2011; Abulhamayel *et al.,* 202; Al Yamoor *et al.,* 2021; Wong *et al.,* 2021 and Cheung *et al*., 2021).

Based on the highly importance of reaching a suffient cleaning and eradication of Enterococcus faecalis bacteria during root canal treatment, the current study highlights the antibacterial efficiency of 980nm Diode laser and 2940nm Er: YAG laser (PIPS) irrigants activation contrasting the technique to side-vented needle irrigation on root canals infected with *E. faecalis* biofilms.

2. Materials and Methods

2.1. Selection and preparation:

Forty-five teeth were collected, all teeth were decapitated at C.E.J, the root lengthwas standardized to be 14 ± 2 mm. Canal preparations were done utilizing ProTaper universal rotary NiTi instruments (Dentsply-Maillefer, Ballaigues, Switzerland), starting from S1 till F4. Using X –smart motor at 300 rpm/ Ncm. (Dentsply, Switzerland), between every file, 2 ml irrigation was used (2.5% sodium hypochlorite solution.

After instrumentation, a total of 3 mL,2.5 % NaOCl along with 3 ml of 17% EDTA was used for 3 minutes for smear layer removal. Then an eventual wash of 3 ml saline were used. All canals were dried by paper points (PP) (Bago *et al.,* 2013).

2.2. Sterilization of specimens:

After completion of cleaning and shaping and before application of different final irrigation protocols, light cured resin composite was used to seal apical foramens in all specimens followed by glazing with double layer of nail varnish to avoid the leakage of bacteria, then were inserted into sterilization bags and underwent sterilization using autoclave at 120c and 1.5 atm (atmospheric pressure) for 20 minutes (Mathew *et al.,* 2014; Bago *et al.,* 2013 and Korkut *et al.,* 2018).

2.3. Confirmation of specimen sterilization:

Sterile saline solution was injected inside the root canal, then sterile PP was introduced into the canals for one minute until it was fully saturated and soaked the saline from the canal. Then the paper point was inserted to test tube contain 1ml of brainheart infusion broth and vortex for 30 seconds. 20 micron of brain heart infusion broth from the tube was taken by sterile single channel micropipette. The broth was spreadon the surface of K.F *streptococcus* agar plate using sterile biological lope.

After culturing the plate was aerobically incubated at 37^0C for 2 days. The culture media were inspected for growth of the bacteria, if the organism had not grown that indicated culture negative (Mathew *et al.,* 2014; Bago *et al.,* 2013 and Korkut *et al.,* 2018).

2.4. Inoculation of specimens with bacteria:

Overnight cultures of E. *faecalis* "ATTCC 29212" grown on bile Aeusculin agar plate"Oxoid, United Kingdom" were used in the study after incubation at 37°c for twenty four hrs. Colonies of E. *faecalis* were picked up by a sterile bacteriological loop and emulsified in streptococcus agar plate. The sterilization bags containing the specimens from all groups were reopened, and the specimens were held by sterile gloves. Utilizing a sterile pipette each specimen was immersed in a 20-microliter volume of a standardized suspension of *Enterococcus faecalis*. The infused specimens from all groups were then placed inside a closed Eppendorf tubes, placed in a rack, and incubated at 37°C for one week. Subsequently, different final irrigation protocols were performed (Mathew *et al.,* 2014; Bago *et al.,* 2013 and Korkut *et al.,* 2018).

2.5. Technique of activated agitation and classification of experimental samples:

Experimental samples were randomly assigned to three primary groups (n = 15) in accordance to the last irrigation protocol:

- Group I (lateral-vented needle agitation): samples were exposed to passive placement of the irrigant using 'lateral-vented needle' (Monoject, Sherwood Medical, Switzerland) inserted 1 millimeter shorter than the working length. For each 1 ml of irrigant the needle was moved in an up- down motion for 10 seconds. Repeating this action four times to a total volume of 4 milliliters.
- Group II (980 nm Diode Laser Agitation) (Laser Activated Irrigation - LAI): Samples were irradiated by 980 nm diode laser (Wiser Doctor Smile, Italy) utilizing the 400 micrometer fiber, positioned less than the full working length by 2 mm. The 2 W laser was intiated for 10-second interval per ml of irrigant. The procedure was replicated four times, each time utilizing 1 milliliter of irrigant and activating the laser for 10 seconds, yielding an irrigant volume of 4 milliliters and a cumulative laser activation period of 40 seconds.
- Group III utilized a 2940 nm Erbium:YAG laser (Fotona, Ljubljana, Slovenia) : set at a frequency of 15 Hz, delivering 20 millijoules of energy per pulse at a power output of 0.3 watts. A 400 micrometer quartz PIPS tip (Fotona, Ljubljana, Slovenia) was employed, the distal three millimeters of the polyamide sheath removed to expose the active tip. The tip was positioned coronally and kept stationary through-out the irrigation process. The concentric air-water spray was deactivated to prevent interference with the laser's effects.

All specimens were irrigated with distilled H2O2 as a final irrigation, then sterile PP were used for canals dryness. Every group was further subdivided into two equally sized subgroups according to the evaluation method, antibacterial activity by CFUs (A) (n=7) and confocal laser scanning microscope (CLSM) (B) (n=7) measuring percentages of living and dead bacteria, and 1 specimen was used in each group as a control group where it did not receive any treatment.

2.6. Methods of antibacterial evaluation:

2.6.1. Colony forming unit (CFUs) (Subgroup A):

After applying different final irrigation protocols, in the presence of distilled water, eachspecimen was activated with K-file size 10. Sterile absorbent paper points (3 successive PP) were inserted into the root canals for 1 minute (Mathew *et al*., 2014).

The three paper points for each specimen were added to tube containing brain heart infusion broth, tubes having the PP were subjected to vortexing for 60 seconds to enable the release of the bacteria into the transporting medium. Serial dilutions (10-folds) were then prepared for every sample. An aliquot of 20 microliters from each dilution was inoculated onto the surface of K.F. *Streptococcus* agar plates and spread evenly using glass rods. The plates were subsequently incubated at a temperature of 37 degrees Celsius for a duration of 24 hours.

2.6.2. Confocal laser scanning microscope (CLSM) (Subgroup B):

After completion of cleaning and shaping and before application of different final irrigation protocols each of the specimens, the roots were sectioned to detect viable and dead bacteria inside the root canal walls. Each root was carefully embedded within acrylic resin blocks, 2 lines were marked

on the resin block to serve as reference points for subsequent analysis (buccal and palatal lines) to guide the vertical sectioning into 2 halves using a microtome saw. After sectioning, each specimen was reassembled using orthodontic wire, light cured composite was applied to seal the apical foramens in all specimens. The specimens were then painted with double nail varnish layers to avoid bacterial leakage, followed by bacterial inoculation of the specimens.

After applying different final irrigation protocols, each of the specimens was disassembled. Acridine orange and Propidium iodide dyes were used individually to stain all experimental samples prior to the CLSM examination. The samples were inspected using Zeiss LSM 710 confocal microscope (Carl Zeiss, Germany) equipped with a 40x objective lens. Three arbitrarily areas at the middle thirds of each RC were selected to be scanned at a step size of 2 millimeters using CLSM. The median intensity of both green and red fluorescence was evaluated for each image acquired. Green fluorescence, indicative of viable bacterial cells, was differentiated from red fluorescence, which signified nonviable bacterial cells. These values were recorded for statistical analysis (Mathew *et al*., 2014).

2.7. Statistical analysis:

Sample size of 45 (15 per group) was determined to achieve 80% power at 5% significance level. The effect size (f) was calculated using G*Power. Means and standard deviation were determined for each group. To evaluate the normality of the data, both the Kolmogorov-Smirnov and Shapiro-Wilk tests were employed. The normally distributed data were analyzed using one-way ANOVA followed by Tukey's post-hoc test ($\alpha = 0.05$) in IBM SPSS Statistics Version 20.

3. Results

3.1. Colony Forming Units (CFU) results:

Group I:

Significant statistical difference was found among the Pre-activated and Post-activation groups ($p<0.001$). The greatest mean value was related to the Pre-group with log10 values (6.598± 0.086), whereas lowest mean value was observed in post activation samples (5.173 ± 0.398) with percentage of bacterial reduction (95.21%). Table (1, 2), Fig (1, 2).

Table 1: The standard deviation (SD) and mean values of log10 values of antibacterial activity of different groups.

Variables	Antibacterial activity				*p-value*
	Pre		Post		
	Mean	SD	Mean	SD	
Group I (Lateral vented needle)	6.598[a]	0.086	5.173[a]	0.398	<0.001*
Group II (Diode laser)	6.648[a]	0.050	3.950[b]	0.356	<0.001*
Group III (Er-YAG laser)	6.600[a]	0.089	2.090[c]	0.697	<0.001*
p-value	**0.275ns**		**<0.001***		

Mean values with different letters are significantly varies from each other.

Table 2. The mean, standard deviation (SD) values of CFUs percentage ofreduction and CLSM of different groups.

Variables	CFUS Percentage of reduction		CLSM			
			Living bacteria %		Dead bacteria %	
	Mean	SD	Mean	SD	Mean	SD
Group I (Lateral- vented needle)	95.210%^{c}	3.047	89.21%^{a}	2.44	10.79%^{c}	2.44
Group II (Diode laser)	97.870%^{b}	2.646	22.95%^{b}	4.84	77.05%^{b}	4.84
Group III (Er-YAG laser)	99.794%^{a}	0.224	6.25%^{c}	1.13	93.75%^{a}	1.13
p-value	**<0.001***		**<0.001***		**<0.001***	

Mean values with different small letters in the same column indicates a significant difference*; significant ($p<0.05$)

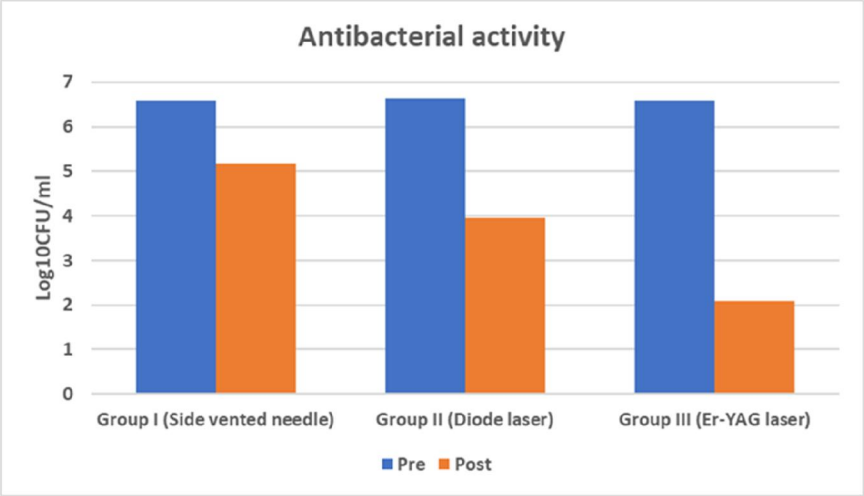

Fig 1: Bar chart representing percentage pre and post results

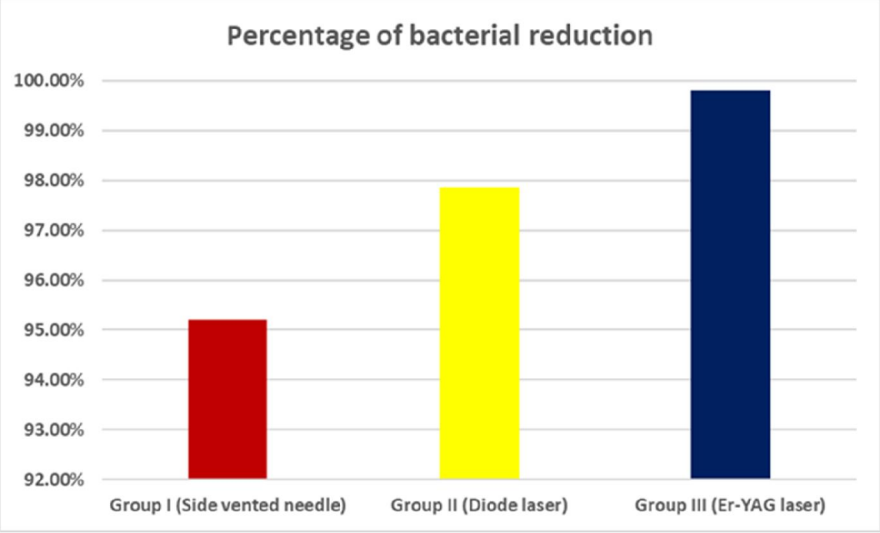

Fig 2: Bar chart illustrating percentage of reduction of different groups

Group II:

A significant statistical difference was observed across the pre-activated and post activated groups, with a p-value of <0.001. The greatest mean value was observed in the preactivated group ,

with log10 values of (6.648± 0.050). On the other hand the lowest mean value was found in (Post) group (3.950 ± 0.356), with percentage of bacterial reduction (97.87%). Tables (1,2), Figures (1,2).

Group III:

Significant statistical difference was found among the preactivated group and post activated group with a p-value of <0.001. The greatest mean value was detected at the (Pre) group with log10 values (6.600± 0.089), whereas the lowest mean value was recorded by (Post) group (2.090 ± 0.697), with percentage of bacterial reduction (99.79%). Table (1,2), Figures (1,2).

A significant statistical difference was observed within the three groups with the p- value was <0.001. The (Er-YAG) group showed statistical difference with both the lateral vented needle and Diode groups with the p-value was <0.001. Furthermore, significant statistical difference was detected between the groups of lateral vented needle and diode. (p=0.026).

CLSM results:

Group I:

Group I (Side vented needle) showed the highest mean value of living bacteria percentage (89.21%) with the lowest mean value of dead bacteria percentage (10.79%).Table (2), Fig (3), Fig (5).

Group II:

Group II (Diode laser) showed (22.95%) living bacteria and (77.05%) dead bacteria. Table (2), Figures (3-5)

Group III:

Group III (Er-YAG) showed the least mean value of living bacteria percentage (6.25%) and the highest mean value of dead bacteria percentage (93.75%). Table (2), Figures (3-5)

A significant statistical difference among all groups (p<0.001) was observed. The lateral-vented needle group differed significantly from both the diode and Er:YAG groups (p < 0.001). Additionally, the diode and Er:YAG groups differed significantly from each other (p < 0.001) in both living and dead bacterial percentages.

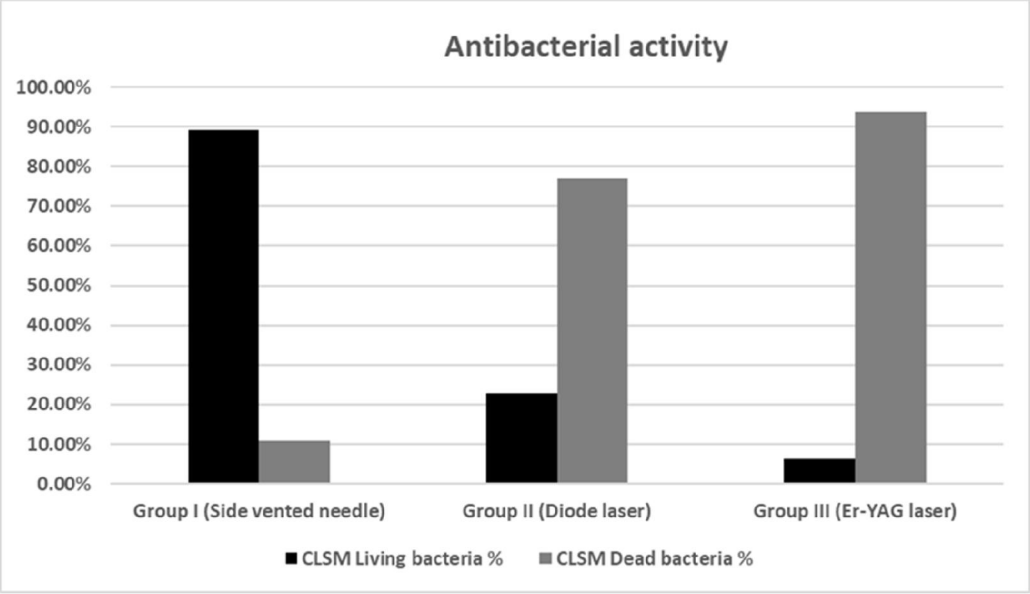

Fig. 3. Bar chart representing CLSM of different groups.

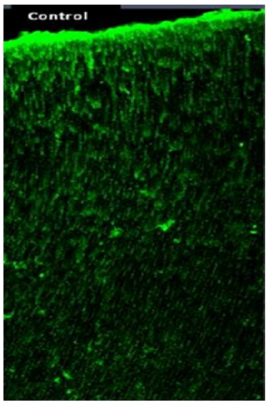

Fig. 4: Antibacterial evaluation under CLSM (Living and Dead bacteria) (Control group)

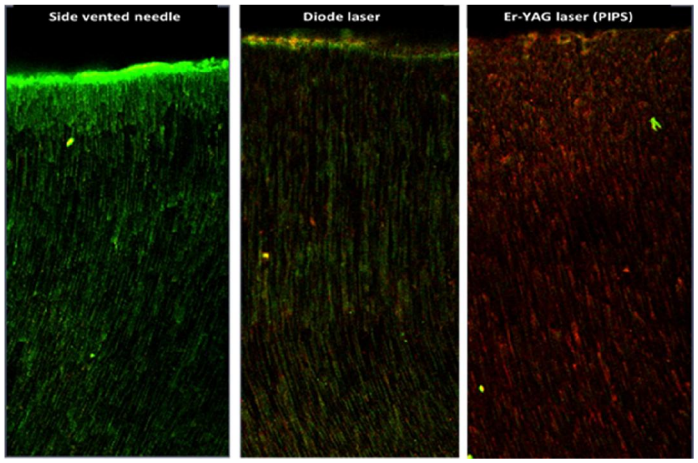

Fig. 5: Antibacterial evaluation under CLSM (Living and Dead bacteria) (Experimentalgroups)

Discussion

Laser irradiation is considered an efficacious tool used for decontamination of root canal system, where lasers supply both thermal energy (Diode) and kinetic energy (PIPS using Er-YAG) to kill bacteria, as we aspire for minimal invasiveness to the root canal system while achieving best cleaning and debridement possible, so the present study focused on the evaluation of the antibacterial efficiency of RC irrigant solutions that was activated by laser utilizing both Diode and PIPS - Er-YAG lasers on infected root canal with *Enterecoccus Faecalis* biofilm.

In the present study rotary instruments (ProTaper Universal) was selected for canal preparation, Protaper Universal instruments were selected because it leaves fewer residual bacteria after biomechanical preparation than manual instruments and significantly produce less canal transportation in the apical portion. However, no significant difference was reported regarding the unshaped areas of the root canal wall (Wang *et al*., 2018). NaOCl, known for its broad antibacterial action and capability to dissolve organic tissue, was used as the irrigant. With respect to the complexity of RC anatomy and limitation of chemical irrigants to effectively disinfect the entire root canal, the use of lasers was considered as a potential method to enhance the outcome root canal treatment (Wang *et al*., 2018).

Previous studies have explored various laser wavelengths for activating irrigants. Laser activation has shown superior efficacy in cleaning root canals compared to traditional methods (De Moor *et al*., 2010).

As an effect of temperature elevation resulting from laser application, coupled with the transfer of pulsed energy to the irrigation solution, contributes to enhanced bacterial killing. In the context of laser-activated irrigation utilizing sodium hypochlorite (NaOCl), the energy delivered by the laser enhances the irrigant's antimicrobial activity by increasing its temperature, which in turn improves the

disinfecting properties, allowing it to better penetrate and clean the RC system. Resulting in efficient bacterial elimination, especially in areas that are difficult to reach (Attiguppe, 2017).

It was revealed that the more generated microbubbles following laser activation within the NaOCl contributed to the enhancement of irrigant efficiency. The microbubbles, resulted due to the rapid heating and cavitation induced by the laser, improving the automated agitation of the irrigating solutions, helping the force removal of debris, and the smear layer with the enhancement of the overall antibacterial action within the root canal (Attiguppe, 2017). It is widely acknowledged that one of the most resistant bacterial strains encountered in root canals is the E.faecalis. Consequently, the positive outcomes observed with laser-assisted disinfection methods for this particular bacterium may also yield improved results for other types of root canal infections (Wang *et al*., 2018).

Bacterial identification can be achieved through two primary methods: plate culture and molecular polymerase chain reaction (PCR) techniques. The plate culture method, a well-established technique for quantifying bacterial growth and reduction, was employed in this study, as it is commonly used in investigations of bacterial reduction. This method allowed for the enumeration of bacteria found in the root canal, offering the advantage of enabling the growth and subsequent identification of all viable cells in the sample. Nevertheless, one limitation of plate culture is its inability to detect low quantities of viable but non-cultivable bacteria. Despite this, our findings demonstrated that the plate culture method was effective in detecting a sufficient number of bacteria for the purposes of the study (Attiguppe, 2017).

Bacteria located deep within the root dentin remain shielded from traditional instrumentation and irrigation procedures due to the limited penetration depth of irrigating solutions. This has prompted the exploration of alternative disinfectants to target endodontic pathogens like E. faecalis. The diode laser has emerged as a promising tool in root canal disinfection. Its penetration depth can reach up to 1000 μm within the dentinal tubules, contributing to its superior bactericidal effects. Diode laser irradiation, with its unique characteristics, allows for deeper penetration into dentin, thereby improving antimicrobial efficacy. Additionally, the thermal photo-disruptive action of the diode laser in dentin further amplifies its bactericidal impact within the root canal system (Mehrvarzfar *et al.,* 2011; Abulhamayel *et al.,* 2021; Al Yamoor *et al.,* 2021 and Wong *et al.,* 2021).

The application of high-power diode laser irradiation led to a significant reduction in E. faecalis colonies in the infected specimens. Consistent with our findings, Thomas *et al.* (2017) reported that diode laser application effectively removed the smear layer and debris from root canal walls. This not only enhances the disinfection properties of the treatment but also induces structural changes in the dentin, contributing to improved overall outcomes in root canal therapy.

In the current study, the diode laser demonstrated higher level of antibacterial action with E. faecalis, this finding is consistent with the results reported by Mehrvarzfar *et al*. (2011), reporting that laser treatment exhibited greater antimicrobial efficacy compared to NaOCl alone. However, the clinical application of diode laser may pose certain challenges, particularly regarding safety concerns. The high temperatures generated during laser use, especially at elevated settings, could potentially damage the tooth structure, making its use in certain cases less practical (Mehrvarzfar *et al*., 2011; Abulhamayel *et al.*,2021 and Al Yamoor *et al*., 2021).

Laser-induced bacterial eradication occurs through thermal heating, both in the surrounding environment and within the bacterial cells, reaching high temperatures enough to cause cell death. The Er:YAG laser, in conjunction with PIPS-activated irrigation and a specialized tapered and stripped tip, facilitated the effective penetration of energy into the root canal system, resulting in a significant reduction of bacterial counts. Unlike traditional thermal mechanisms, this technique relies on photomechanical effects, using laser energy to disrupt bacterial cells more efficiently, resulting in a significant decrease in bacterial load within the root canal (Korkut *et al*., 2018).

A study in Korkut *et al.,* (2018) highlighted the combined outcome of Er:YAG laser- PIPS-agitated irrigation, with NaOCl in reducing microbial populations. This effect can be attributed to two main mechanisms: firstly, is the significant reaction of NaOCl, leading to an increase in the available chlorine content. Which is potent oxidant, exhibits antimicrobial properties by targeting bacterial enzymes, causing irreversible oxidation of sulfhydryl groups. Secondly, the enhanced movement of particles induced by the Er:YAG laser, which improves the interaction between active chlorine molecules and the microorganisms. This increased contact enhances the bactericidal efficiency of the irrigant, promoting more effective microbial eradication (Korkut *et al*., 2018).

Due to its high surface tension, NaOCl has limited flow, typically reaching only about 130 µm into dentinal tubules, while bacteria can colonize deeper regions. Consistent with the findings of this study, previous research by Korkut *et al.* (2018), demonstrated the effective use of Er:YAG laser radiation with PIPS-agitated irrigation to eliminate bacteria from various root canal systems, reaching up to 300 micrometers depth from the pulpal end of the root canal. This deeper penetration highlights the enhanced disinfection potential of laser-assisted irrigation techniques (Korkut *et al*., 2018). The photomechanical streaming effect of NaOCl, involving the formation of microbubbles within the fluid, helps the irrigant reach otherwise inaccessible areas within the root canal more effectively. This dynamic process improves the penetration of NaOCl into complex canal systems. Additionally, it had shown that increasing the activation time can further enhance the penetration depth of the irrigant. Addition of surfactants to NaOCl, can also contribute to improved penetration and microbial reduction by altering the fluid's surface properties (Korkut *et al*., 2018).

Thus, in accordance with many previous studies on comparing the antimicrobial efficiencyof both Diode laser and Er:YAG laser activation with PIPS irrigant activation, on E. faecalis biofilms, both lasers activation led to a marked reduction in the colony count of E.faecalis in the infected samples, both lasers were superior in reducing the overall biomass although the maximum percentage of dead bacteria wasfound when NaOCl was activated by PIPS (Korkut *et al*., 2018; Suer *et al*., 2018 and Al Shahrani *et al*., 2014).

The CFUs assessment was considered insufficiently sensitive to identify the viable bacteria at low concentrations. In contrast, the Confocal laser scanning microscopy analysis (CLSM) employed in the current study offered a more reliable, accurate, and reproducible technique for localization and quantification of both live and dead bacteria within dentinal tubules. This approach provides valuable insights into the extent of dentin infection and the viability of bacteria at a cellular level. CLSM is particularly useful for investigating the presence of residual bacteria in an uncultivable state, offering a clearer understanding of the bacterial persistence within the root canal system (Mathew *et al*., 2014)

In this study, percentage of viable and non-viable bacteria were detected in the samples of all groups at middle segment of the root. Each irrigation protocol showed differenteffectiveness in killing bacteria. PIPS provided a high antibacterial performance with themaximum bacterial reduction, with the highest percentage of dead bacteria compared to percentage of live bacteria as showed by CLSM results, followed by Diode laser. Onthe contrary, Side vented needle showed the lowest percentage of dead bacteria and the highest percentage of live bacteria. This may stem from the decreased irrigant penetration on using Side vented needle subsequently decreasing in its disinfection ability than both Diode and PIPS irrigation protocols.

5. Conclusion

The current in-vitro study findings recommend:

- Diode and Er:YAG lasers had better disinfection than conventional irrigation methods (Side vented needle).
- The use of Er:YAG laser (PIPS) for activating irrigants resulted in improved depth of the irrigants, with subsequently more bacterial eradication and disinfection with minimal thermal effect and less viable bacteria.

Ethical approval:

The current study received approval from the Ethics Committee of the National Institute of Laser Enhanced Science (NILES), Cairo University (Ethical Code: 019/029).

Conflict of Interests No conflict of interest.

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