Evaluation of efficacy of photodynamic disinfection against *enterococcus faecalis* and compare it with the effect of different endodontic irrigant activation techniques.

Amanda George Haskiyal [(1)], Dara Hamarashid Saeed[(2)]

Background and objective: The aim of this in *vitro* study was to assess the efficacy of photodynamic disinfection against *Enterococcus faecalis,* and compare it with the effect of different endodontic irrigant activation techniques.

Methods: Fifty single-rooted premolars with single canals were chosen for the study. Five teeth were chosen to serve as the negative control group. The remaining samples were infected with normal *Enterococcus faecalis* strains. They were then randomly separated into five groups, with nine teeth serving as a positive control group (E. Cont.). Each group consisted of 9 samples based on the kind of irrigant activation: E.A (Diode laser), E.B (Ultrasonic), E.C (conventional), and E.D (FotoSan®). The ProTaper NEXT rotary system up to model X3 was used to prepare all samples. For each group, 5.25% NaOCl was injected as an initial irrigant with a 30G double-side vented syringe needle, followed by 17% EDTA, then activated with NaOCl as a final irrigant. CFU of each sample was counted before and after irrigation regime, and they were compared to the control group. The efficiency of the disinfection techniques was determined using scanning electron microscopes (SEM).

Result: The quantity of bacterial colony counting revealed that the control group had the largest mean of a bacterial colony (135.56±50.58); while the FotoSan group had the lowest colony mean number (23.11±9.82). SEM results indicated that the FotoSan group (5.56±3.16) and Diode laser (5.56±3.57) had the lowest surviving colony mean number.

Conclusion*:* As an additional method, photodynamic therapy may increase the disinfection capacity of traditional endodontic treatment against *E. faecalis* in the root canal.

[(1)]Department: Conservative Dentistry, College: College of Dentistry University: University of Duhok, City: Duhok, Iraq.
[(2)] Department: Conservative Dentistry, College: College of Dentistry University: Hawler Medical University, City: Erbil, Iraq.
Corresponding author: Amanda George Haskiyal, mandy.ghb@gmail.com.

Introduction

Endodontic therapy's primary goal is to minimize any threat to treatment effectiveness by creating a bacteria-free environment in the root canal system. Endodontic infection removal varies from infection removal in most other parts of the human body. Because root canals have a unique structure and physiology, host attempts to remove the infectious organism from other places are insufficient to totally eradicate endodontic infection.[1]. The root canal system is completely debrided utilizing a combination of mechanical shaping with endodontic tools and chemical disinfection of root canal systems using the various irrigation chemicals and activation procedures available.[2].

Endodontic infections are polymicrobial, with complex bacterial interactions and anaerobic species predominating. A heterogeneous consortium of around 600 microorganisms organized in biofilms. *Enterococcus faecalis* has been identified as one of the most prevalent microorganisms found in infected root canals and retreatment patients.[3]. They are the most typically involved bacterium in both asymptomatic and symptomatic chronic infections. Endodontists have significant difficulty due to the organism's complicated nature. The failure rate for post-treatment apical periodontitis ranges from 24% to 77%, with *Enterococcus faecalis* being responsible for up to 77% of therapeutic failures.[2].

Endodontic therapy now includes a wide variety of irrigation treatments. Dentinal tubules penetration, antibacterial activity, smear layer removal, stability, and substantive efficacy are all being enhanced in their formulations. None of the irrigation options appear to possess all of the desired characteristics.[4]

Before to the invention of sonic and ultrasonic activation techniques, conventional syringe irrigation was supplied as an effective and readily available method of irrigation. However, several studies have found that the irrigant solution has only a limited influence beyond the needle tip due to dead water zones or even air bubbles in the apical canal segment; hence, irrigant apical penetration is hampered .[9]

Various mechanical devices have been developed during the last few decades to enhance irrigation penetration and efficacy in the root canal space. Endoactivator, a sonic tool, is used to vigorously agitate the irrigant solution in the root canal. Removing the smear layer and dislodging clumps of simulated biofilm may also assist in deeper penetration of an irrigant to all parts of the endodontic space and successfully remove debris from the lateral canal.[6] According to research, ultrasonic activation can improve accessory canal cleanliness in the apical third of the root canal system and increase the depth of irrigating solution penetration into the dentina.[8]

The use of high-power diode lasers and photo-activated disinfection were two new root canal disinfection approaches (PAD). In comparison to chemical disinfectants, which have a depth of penetration of just 100 μm, the Diode laser has a stronger bactericidal impact irradiation and a greater depth of penetration (up to 1000 μm into dentinal tubules). Irrigant penetration is impeded by the increasing narrowing of the diameter of deep dentinal tubules.[7]

When a dye, acting as a photosensitizing agent, absorbs photons from a light source, its electrons reach an excited state, also known as the triplet state. When a substrate, such as oxygen, is present, the photosensitizer returns to its basic state and transfers energy to the substrate, resulting in the formation of extremely cytotoxic free radicals such as superoxides and singlet oxygen.[10] By permanently oxidizing cellular components, these very reactive molecules can cause significant injury to bacteria, resulting in damage to the cell membrane, mitochondria, nucleus, and other microbial cell components. It is an antibacterial device that uses low-level laser light to activate a harmless photosensitizer as well as the nascent oxygen created by dyes. It is used in root canal treatment as an alternative or addition to disinfection techniques. [6]

Methods

Fifty single-root premolars with single canals were chosen to be used in this research. Those teeth had just been pulled for orthodontic purposes. Caries, root resorption, severe curvature, root fracture, operational therapy, and fractures were not present. To prevent dryness of the tooth root surfaces, they were stored in a container filled with normal saline. A periodontal scaler was used to mechanically clear them of tooth calculus and soft tissue remnants. The samples were then prepared to be decoronated with a diamond disk and confirmed with an electronic caliper to have a length of 12 mm coronally to the apex. The canals' patency was then confirmed using a file 10 k-file. And the working length was fixed to 11 mm after the file point was extruded beyond the apical foramen. Following confirmation of the working length of each sample. Before sculpting the canals with Protaper Next files, the foramen was extended to k-file #10 to create a standardized foramen.

Following root canal preparation, the expanded apical foramina were sealed with flowable resin composite cured with a cordless curing light to minimize bacterial leakage, avoid solution extrusion beyond the apex, and imitate the natural environment of the tooth inside the oral cavity.[11]

Molds for holding the teeth were made from a water pipe cut to (22 mm height) and a heavy body impression combined with a catalyst. Each pipe was filled with heavy body silicone that had been applied to it. The samples were carefully put in the impression material in the pipe before it was set, leaving the coronal section of the root exposed (2mm). The pipes were then left in place for the impression to take ef-

fect. The silicon impression was molded in the pipe that had previously been prepared in order to achieve a uniform location and orientation of canal preparation. The samples were randomly separated into five groups based on the final irrigation that will be employed later. Those molds were secured to a table with the help of a table jack that was secured to the table. Each group had ten teeth chosen at random. All of the teeth and their associated pipes were coded and secured to the table.

Each sample was instrumented using Protaper NEXT fifth-generation rotary files (Dentsply Maillefer, Ballaigues, Switzerland) and an NSK ENDO-MATE Endodontic Motor (TC2 Japan). accordance to the manufacturer's recommendations, by a series of X1, X2, and X3 at 300 rpm and 3.5 Ncm A 10# K-file was used to recapitulate each ProTaper NEXT rotary file sequence. Irrigation solution was also administered between each endodontic rotary file using a 30-G Double side-vented endodontic irrigation needle and 2 ml of 5.25% NaOCl (Chlora XiD, Cerkamed, Poland).

After decoronation and instrumentation, the root samples were soaked in EDTA for 1 minute before being placed in the ultrasonic tub. The samples were then immersed in 5.25% NaOCl for 1 minute before being placed in an ultrasonic tub to sanitize the surface of the roots. They were then immersed in pure distilled water .[12]To maximize disinfection, each root canal was irrigated with 2 ml of 5.25% NaOCl and then with normal saline to eliminate the impact of the NaOCl. Those samples were thoroughly dried using paper point X3.

Before placing the samples in the sterilizing pouches, they were all covered in aluminum foil. To minimize cross contamination, all samples were placed in separate pouches. After all of the samples were sealed with sterilization pouches, they were placed in the autoclave for 45 minutes at 121 °C under 1.5 psi pressure to verify that no microorganisms remained.[13] Five samples were chosen at random and inculated on Muller Hinton agar to guarantee full sterilization.

the first part of the study, the 12 specimen's discs were divided into two equal subgroups to simulate two different methods of fluoride application to the materials (Transbond plus color change, Resilience, Transbond XT), namely, brushing with a fluoride-containing toothpaste or gel application.

Vitek 2 (bioMérieux, Inc, Hazelwood, MO) bought and confirmed standard strains of Enterococcus faecalis (ATCC 29212). After isolation, a single colony of E. faecalis from a 24-48-hour culture was chosen and put in Brain Heart Infusion (BHI). This was inoculated for 48 hours at 37° C in an incubator. After incubation, bacteria were serially diluted to achieve the appropriate colony forming unit (CFU)/ml per plate concentration. A series of dilutions were carried out till 1/100000 was obtained. Using a sterile micropipette, each sample was infected with 10 µL of prepared *Enterococcus faecalis* and incubated for 48 hours at 37° C. After incubation, the control group was swapped and cultivated on Mueller Hinton agar plates to be compared later with the samples that would be disinfected.[14]

The forty-five infected teeth were randomly split into five groups of nine samples each. Nine samples were chosen to serve as the control group, which means they did not go through any disinfection procedures. The control group's swabs were then grown on Mueller Hinton agar. Following the completion of the inoculation onto the Mueller Hinton agar culture, the CFU of the control group was counted, and the mean value for *Enterococcus faecalis* was 135 X 10^7, which was subsequently utilized as a standard quantity of CFU for comparing the samples to the control group. The sample groups were as follows:

Group E. Cont.: Control Group: 9 teeth without irrigation.

Group E. A: Ultrasonic group: Each root canal was irrigated with 4 ml of 5.25% NaOCl solution. The irrigation suspended 1 ml of sodium hypochlorite 5.25 % NaOCl, then 1ml of 17% EDTA for 2 minutes. Followed by 1ml of NaOCl that was ultrasonically activated for 20 second per cycle by using a woodpecker ultrasonic system with tip E12 (Ufile size 25). The cycle was repeated for three times. NaOCl was refreshed at each cycle by 1 ml

Group E. B: Diode laser group: Each root canal was irrigated with 4 ml NaOCl solution (5.25%) that was finished in 2 minutes.

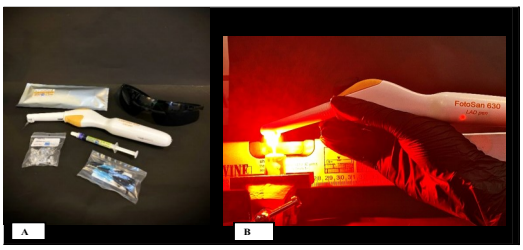
NaOcl 5.25% was agitated with 940 nm diode laser, Biolase iLase diode lasers, the delivery was by fiber-optic endodontic tip, E2 with the tip diameter of 200 µm. Specimens were irradiated with 1.08 W, CW mode; the fiber tip was inserted 2 mm from the apex, in contact mode, and helicoidal movement in a speed of 1mm/s (by hand training) from apical to cervical direction (Figure 2). This was accomplished in 18 s and repeated two times resulting in a total irradiation time of 36s for each sample according to manufacturer instructions.[15]

Group E. C: Conventional group: Each root sample was irrigated with 4ml of NaOCl 5.25% by using 30G side vented needle was fixed to the syringe. 3ml was finished, followed by 1ml EDTA then again flushed by 1ml of NaOCl 5.25% (ChloraXiD).

Group E. D: FotoSan group: Same procedure was applied as a conventional group followed by dryness of the samples prior to photosensitizer application. Shaking of the gel that was in a syringe with a suitable needle before filling the canals with the solution. The canals were filled with 0.1 mg/ml of the active ingredient, toluidine blue, low viscosity of the FotoSan agent for 60 seconds. Stirring of the solution inside the canals a couple of times to avoid creating air bubbles by using a small size hand file at working length. The LED light source (FotoSan®; CMS Dental; Denmark), produces red light with a wavelength of 630 nm and intensity 2000 mw/cm2 for 30 seconds, (Figure 1).[16]

Figure 1: A: FotoSan® device. B: Photo-activation procedure

All canals were flushed with normal saline



before being dried with two X3 paperpoints. Protaper Next (Dentsply, Maillefer, Switzerland), the first was left for 10 seconds, and the second was to collect a swab, which was then grown on Muller Hinton agar plates and incubated for 48 hours at 37 °C. After two days of incubation, the CFU was counted and compared to the control group using a colony counting device. The culture plate was deposited in a special waste bag once each sample's colony counting was completed. The samples were then prepared for imaging using scanning electron microscopy. Each sample was processed for SEM by cutting the roots longitudinally in the buccolingual plane. To produce a guide for cutting the roots into two equal halves, all roots were grooved longitudinally on the exterior surface using a diamond disk. The diamond disk was then placed on those guides to cut through the grooves without reaching the interior canal. The samples were dehydrated with varying concentrations of ethyl alcohol, and mounted on coded stubs, and the canal surface was sputter-coated with a 300 Angstrom gold layer before being inspected under SEM at magnifications ranging from X3000-X5000.[17]

Results

Shapiro-Wilk test (Table 1) indicated that the data follow the normal distribution, because all PVs are greater than 0.05. In this study, the control group CFU/ml was 135 X 10^7before other samples underwent irrigation. The results of bacterial colony counting showed that the highest mean of the bacterial colony was found in the control group (135.56±50.58); while the lowest colony mean number was found in FotoSan group (23.11±9.82).

Furthermore, the results showed that Diode laser (34.78±24.54), ultrasonic (37.22±17.11) and conventional groups were intermediate between control and FotoSan groups, with the conventional group (62.78±20.27) having higher colony number from Diode laser and ultrasonic group (Table 2). This is clearly shown in the bar chart below (Figure 3).

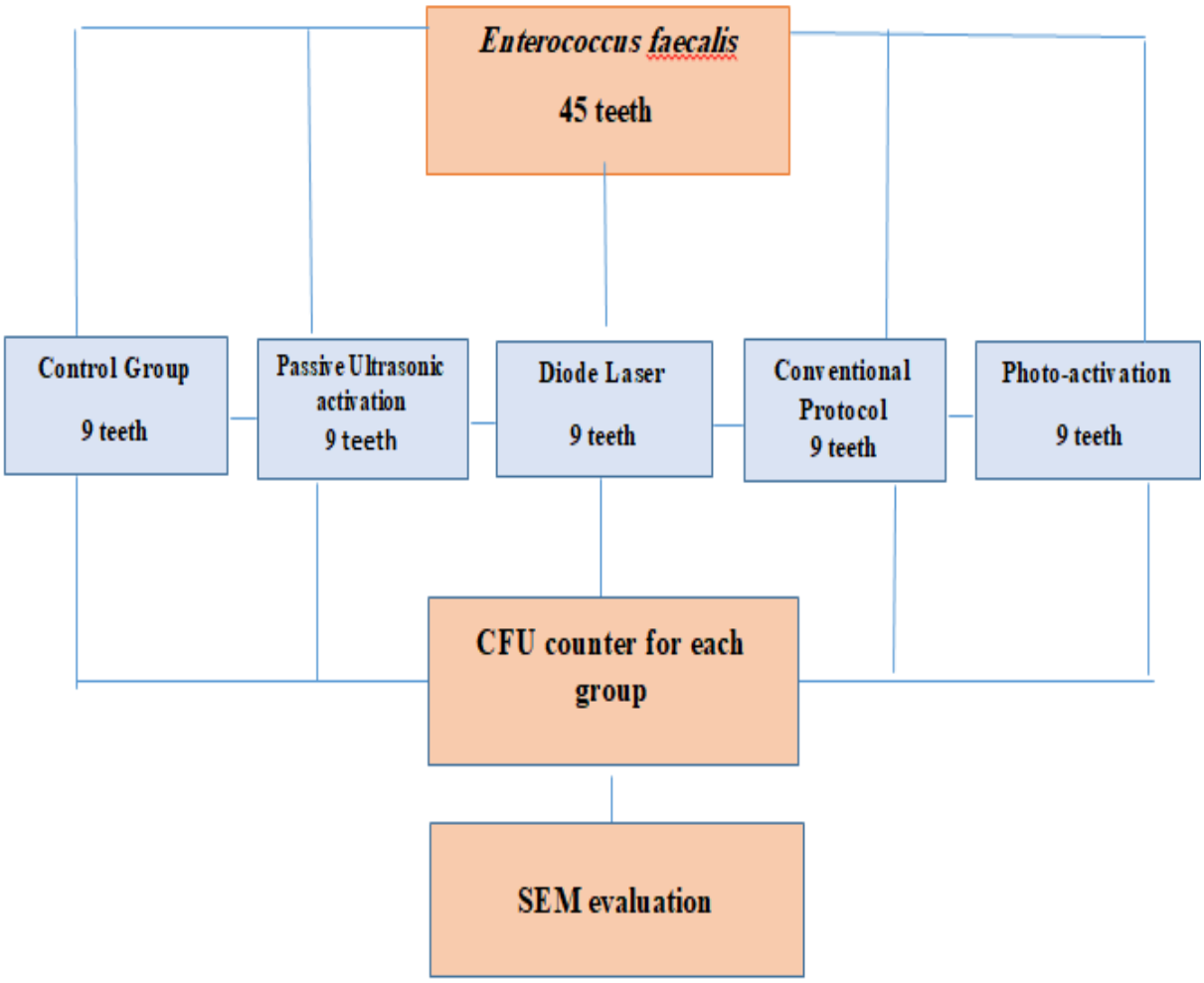


Figure 2: Study design for Infection and disinfection method.

Table 1: Shapiro-Wilk

Group	Shapiro-Wilk		
	Statistic	df	PV
Control	0.969	9	0.890
Ultrasonic	0.902	9	0.263
Diode Laser	0.927	9	0.452
Conventional	0.851	9	0.076
FotoSan	0.891	9	0.203

Table 2: Descriptive statistics of bacterial colonies reduction of the five groups

Group	Min	Max	Mean	Standard Deviation
Control	81.00	205	135.56	50.589
Ultrasonic	15.00	71	37.22	17.116
Diode Laser	10	77	34.78	24.545
Conventional	40	101	62.78	20.278
FotoSan	11	38	23.11	9.829

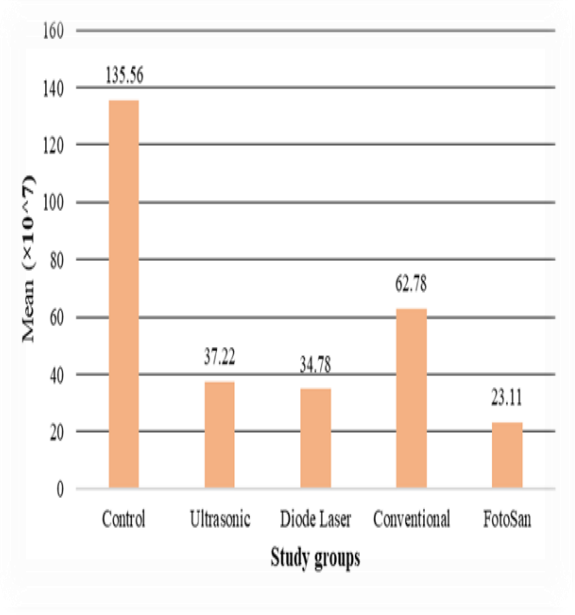


Figure 3: Bar chart graph showing the mean values of bacterial colony units.

Identifying the source of substantial differences between groups Post Hoc Tests (Tukey) were done, and it was discovered that there was a substantial difference between the control groups (Diode laser, ultrasonic, conventional, and FotoSan) and the FotoSan and conventional groups. However, there was no significant difference between ultrasonic with (Diode laser, conventional, and FotoSan) and Diode laser with (conventional and FotoSan); (Table 4).

The remaining bacteria confirmed by SEM revealed that the highest mean of the remaining bacterial colony was found in the control group (25.33±9.82), while the lowest remaining colony mean number was found matching in the FotoSan group (5.56±3.16) and Diode laser (5.56±3.57), with a small difference in standard deviation value. Furthermore, the results revealed that the ultrasonic (8.89±3.75) and conventional groups had

had the same mean value as the control and FotoSan groups. Despite the fact that the conventional group (8.89±4.88) has a the ultrasonic group. The bar chart below clearly demonstrates this. (Figure 4).

Table 3: One-way ANOVA test of bacterial colony reduction between groups

Sources of Variation	Sum of Squares	df	Mean Square	F	PV
Between Groups	74011.867	4	18502.967	23.348	<0.001
Within Groups	31699.778	40	792.494		
Total	105711.644	44	-	-	-

Table 4: Post Hoc Tests (Tukey) among the groups

(I) Groups	(J) Groups	Mean Difference (I-J)	Std. Error	PV
Control	Ultrasonic	98.333**	13.271	<0.001
	Diode Laser	100.778**	13.271	<0.001
	Conventional	72.778**	13.271	<0.001
	FotoSan	112.444**	13.271	<0.001
Ultrasonic	Diode Laser	2.444	13.271	1.000
	Conventional	-25.556	13.271	0.321
	FotoSan	14.111	13.271	0.824
Diode Laser	Conventional	-28.000	13.271	0.236
	FotoSan	11.667	13.271	0.903
Conventional	FotoSan	39.667*	13.271	0.036

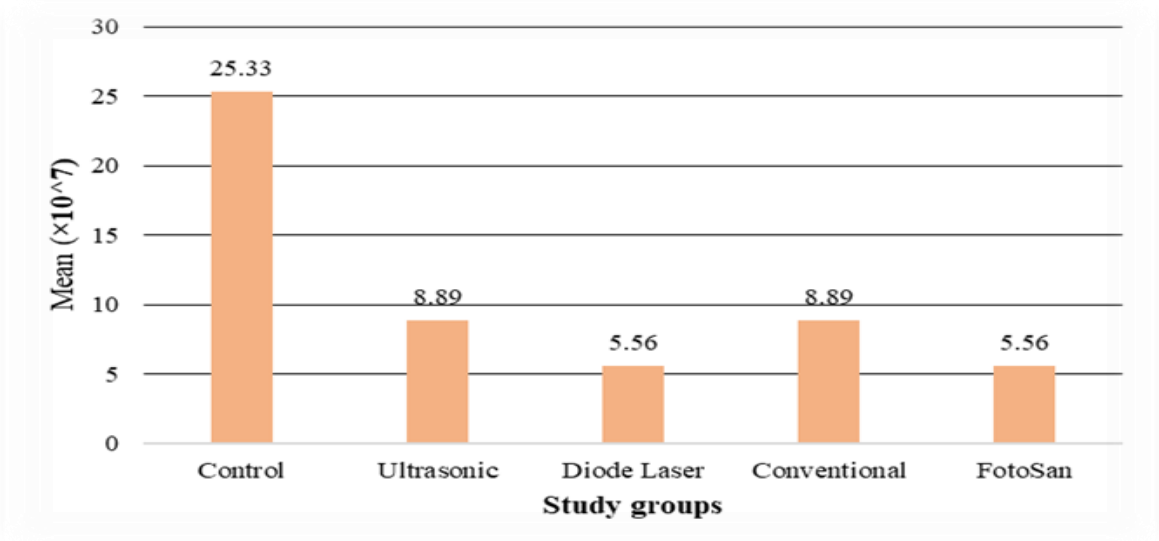


Figure 4: Bar chart graph showing the mean values of the remaining bacteria colony number.

Table 6: One-way ANOVA test of remaining bacterial colony between groups.

Sources of Variation	Sum of Squares	df	Mean Square	F	PV
Between Groups	2461.689	4	615.422	19.565	<0.001
Within Groups	1258.222	40	31.456		
Total	3719.911	44	-	-	-

Post Hoc Tests (Tukey) revealed a significant difference between groups and revealed a significant difference between the control group and the experimental group (Diode laser, ultrasonic, conventional and FotoSan). Nonetheless, there was no statistically significant difference between ultrasonic with (Diode laser, conventional, FotoSan) and ultrasonic with (conventional and FotoSan). Besides the non-significant difference between the conventional and FotoSan groups; (Table 8).

Discussion

Endodontic therapy considers root canal irrigation to be a very significant and necessary aspect of eliminating tissue remnants and bacteria during instrumentation.[18] Bacteria present in the pulp and root canals are the primary cause of periapical and pulpal infections. Root canal treatment is a method of cleaning, disinfecting, and removing microflora and inflammatory pulp tissue, as well as shaping and filling the canal.[19] Until recently, endodontic infection therapy was centered on the non-specific massive killing of endodontic bacteria using broad range of antimicrobials, primarily sodium hypochlorite (NaOCl). Root canal infections are polymicrobial in nature, which means that a variety of bacteria and/or their

Table (8): Post Hoc Tests (Tukey) among the groups.

) Groups	(J) Groups	Mean Difference (I-J)	Std. Error	PV
Control	Ultrasonic	16.444**	2.644	<0.001
	Diode Laser	19.778**	2.644	<0.001
	Conventional	16.444**	2.644	<0.001
	FotoSan	19.778**	2.644	<0.001
Ultrasonic	Diode Laser	3.333	2.644	0.716
	Conventional	0.000	2.644	1.000
	FotoSan	3.333	2.644	0.716
Diode Laser	Conventional	-3.333	2.644	0.716
	FotoSan	0.000	2.644	1.000
Conventional	FotoSan	3.333	2.644	0.716

interactions are involved in the disease's development and progression, either directly or indirectly.[20]

NaOCl has a limited ability to remove the smear layer, which may obstruct adequate cleaning of the dentinal walls and tubules. As a result, a chelating (demineralising) agent is necessary to achieve this \simultaneously.[21]

When comparing the initial subgroups infected with *Enterococcus Faecalis* and incubated for 48 hours at 37 °C in the current investigation. These samples were irrigated and activated, and the findings were compared to a control group in terms of the capacity of different activation regimes against this type of bacteria. The antibacterial impact of four disinfection procedures was studied in vitro and is considered an addition to chemo-mechanical canal preparation. Except for the control group, the four groups were irrigated with 5.25% sodium hypochlorite using a 5mL syringe with a 30-G needle.

In terms of antibacterial ability, photodynamic treatment has been compared to various activation devices. To disinfect the canals, ultrasonic, Diode laser, and conventional sodium hypochlorite was employed, and the colony formation results after the regime were compared to the control group. The statistical analysis of this study revealed that Group E.D (5.25% NaOCl + FotoSan 630®) was the best irrigation and activation regime among the five irrigation regimes utilized in this study. In which the mean value was 23 X 10^7, and his finding is consistent with.[22 23 24]

One advantage of photosensitization over traditional antimicrobials is that because the interaction of highly reactive oxygen with organic molecules is not specific, any macromolecule within a microbial cell may become a potential target, preventing the development of microbial resistance mechanisms. Furthermore, the operation can be done multiple times.[25]

The aforementioned findings are also consistent with prior research, which found that the standard syringe irrigation technique distributes irrigating fluid no more than 0-1mm beyond the needle tip. The needle point is frequently located in the coronal and central portion of the canal. This is because irrigating solutions' depth of penetration and ability to disinfect dentinal tubules are constrained and do not reach peripheral regions such as anastomoses connecting canals, fins, and the most apical section of the main root canal.[24]

However, this study has dissimilarity with the other study,[26] the activation of MB occurs at a distance, regardless of direct contact of the light delivery system with the photosensitizer, and may also be caused by the low concentration of oxygen available in the canals, which led to the conclusion that conventional irrigation regime achieved high percentages of reduction of *E. faecalis* than PDT.

Additionally, the statistical analysis of this study's data showed that there was no statistically significant difference between the reduction of bacterial colonies using a diode laser and ultrasonic technology, with the laser effect coming in second place, followed by ultrasonic and conventional irrigation, respectively. The removal of debris from the irregularly shaped root canals and oval-shaped canals was made easier despite the greater frequency of cavitation leading to increased acoustic streaming and the flow of the irrigant at a high velocity, which was achieved owing to passive ultrasonic activation. When compared to laser irrigation, ultrasonically activated irrigation has demonstrated lower efficacy, and the factors influencing these results may include the placement of the suction, the width of the irrigation jet, the location and size of the root canal orifice, and the amount of irrigant flowing through the canal. Other elements that affect the duration of irrigation and the amount of irrigant. [27]

The irrigant's penetration gets hindered when the size of the dentinal tubules decreases apically. Because of its inherent properties of light scattering, increased local intensity, and attenuation, the laser allows light to penetrate deeper into dentinal tubules, leading to its better antibacterial activity. The diode laser generates thermal photo disruption in the undisturbed dentin, resulting in an amplified bactericidal effect

on the root canal dentin. A contract was discovered with a research raised by.[28]

Images were obtained to supplement the lab results and were then subjected to scanning electron microscopy to verify the results of the disinfection level of the irrigants. In this study, the degree of disinfection of the apical part of the root canal was assessed using magnifications of 3000X and 5000X. Imaging the root canal walls using SEM is one of the best approaches to assess the post-operative root canal assessment.[29] A large load of the injected bacteria and debris in the canal at the apical region could be detected in the SEM pictures for the Control Group *Enterococcus faecalis,* according to the statistical analysis. The similar mean difference between the Foto-San group and the Diode laser was observed in the apical sectioned region that was scanned, and this may have been caused by increased radicular dentin permeability and ultrastructural alterations in the root canal walls. The kind of irrigation solution used and the laser's wavelength affect how well a root canal irrigant absorbs. Light of a particular wavelength activates a photosensitizing molecule linked to the bacterial/fungal membrane, resulting in the generation of highly reactive oxygen species capable of killing microorganisms.[22 23]

Marchesan et al. further said that the process for laser activation of irrigating solutions is generated by the absorption of laser energy by the irrigant solution, the formation of vapor bubbles, and the collapse of the bubbles, sonic streaming, and cavitation. That explains why using laser results in fewer residual bacteria.[30]

Second, the ultrasonic group with traditional irrigation revealed the same mean values that were more bacterial appeared left in comparison to the other groups. As it is difficult to entirely eliminate the leftover bacterium, since the apical third's smaller size compared to the other thirds impedes the circulation and action of the irrigating solutions. Other research has found agreement.[31 32] Ghinzelli et al. underline that using ultrasonic activation on photodynamic treatment boosted its potential for dentinal tubule cleansing.[33]

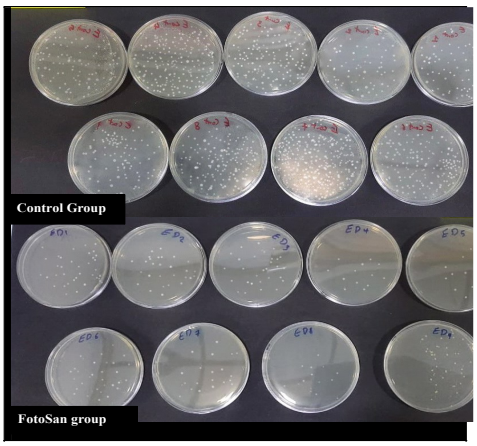


Figure 4: Colony forming unit of control and photoactivated plates sample.

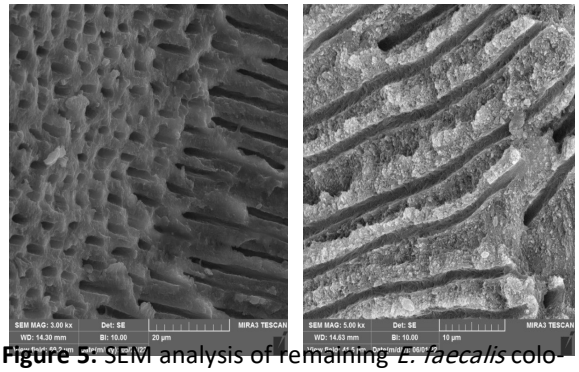


Figure 5: SEM analysis of remaining *E. faecalis* colonization on root canal walls of the samples treated with FotoSan®.

Conclusion

Our findings show that photodynamic treatment is effective as a bactericidal agent in a tooth model contaminated with Enterococcus faecalis, although it does not completely eliminate the contaminating bacteria. As an adjuvant method, it has the potential to boost the antibacterial ability of traditional endodontic therapy against E. faecalis. A longer irradiation duration may

be advised to improve the effect.

Conflict of interest

The author reported no conflict of interests.

Reference:

1.Haapasalo, M., Endal, U., Zandi, H., & Coil, J. M. (2005). Eradication of endodontic infection by instrumentation and irrigation solutions. Endod. topics, 10(1), 77-102.

2.Mashalkar, S., Selvakumar, G., Diwanji, P., Indi, S., & Warad, J. (2019). Apical microleakage in root canal-treated teeth containing broken hand files obturated with two different obturating materials: An in vitro study. *Endodontology*, 31(1), 68.

3.Choudhary, E., Indushekar, K. R., Saraf, B. G., Sheoran, N., Sardana, D., & Shekhar, A. (2018). Exploring the role of Morinda citrifolia and Triphala juice in root canal irrigation: An ex vivo study.: *JCD*, 21(4), 443–449.

4.Walsh, L. J. (2020). Novel approaches to detect and treat biofilms within the root canals of teeth: a review. Antibiotics. *MDPI.*, 9(3), 129.

5.Siqueira Jr, J. F., & Rôças, I. N. (2005). Exploiting molecular methods to explore endodontic infections: part 2—redefining the endodontic microbiota. *J. Endod.*, 31(7), 488-498.

6.Pandey, D., Paliwal, A., Singh, P., Brahmachary, B., & Singh, P. (2022). Antimicrobial Efficacy of Three Different Disinfecting Systems against E. Faecalis A comparative-in vitro study. *JPSP* 6(2), 5415-5421.

7.Hegde, M. N., Bhat, R., & Shetty, P. (2015). Efficiency of a semiconductor diode laser in disinfection of the root canal system in endodontics: An in vitro study. *JICDRO.*7(1), 35.

8. Souza, M. A., Pazinatto, B., Bischoff, K. F., Palhano, H. S., Cecchin, D., & de Figueiredo, J. A. P. (2017). Influence of ultrasonic activation over final irrigants in the removal of photosensitizer from root canal walls after photodynamic therapy. *Photodiagnosis and photodynamic therapy*, 17, 216-220.

9. Boutsioukis, C., Gogos, C., Verhaagen, B., Versluis, M., Kastrinakis, E., & Van der Sluis, L. W. M. (2010). The effect of apical preparation size on irrigant flow in root canals evaluated using an unsteady Computational Fluid Dynamics model. *Int. Endod. J* 43(10), 874-881.

10. Dai, T., Huang, Y. Y., & Hamblin, M. R. (2009). Photodynamic therapy for localized infections—state of the art. *Photodiagnosis and photodynamic therapy*, 6(3-4), 170-188.

11. Balakrishna N, Moogi P, Kumar G V, Prashanth B R, Shetty NK, Rao KR. (2017). Effect of conventional irrigation and photoactivated disinfection on Enterococcus faecalis in root canals: An in vitro study. *J Conserv Dent.* 20(2), 125-128.

12. Race, J., Zilm, P., Ratnayake, J., Fitzsimmons, T., Marchant, C., & Cathro, P. (2019). Efficacy of laser and ultrasonic-activated irrigation on eradicating a mixed-species biofilm in human mesial roots. *Aust Endod J.*, 45(3), 317-324.

13. Mohammadi, Z., & Shalavi, S. (2021). ANTIMICROBIAL ACTIVITY OF QMIX: A REVIEW. *Int. J. Clin. Dent.,* 14(3), 353-358.

14. Cain D., Hanks H., Weis M., Bottoms C., and Lawson J. (2017) Microbiology Laboratory Manual B2420. Collin County Community College District, McKinney, TX.37.

15. Al-Mafrachi, R. M., Awazli, L. G., & Al-Maliky, M. A. (2018). The Effect of using 940 nm Diode Laser in Comparison with Endoactivator on Radicular Dentin Permeability and Smear Layer Removal (An in Vitro Study). *Iraqi Journal of Laser*, 17(B), 9-15.

16. Mittal, R., Tandan, M., & Jain, V. (2020). A randomized clinical trial of antimicrobial efficacy of photoactivated disinfection, conventional endodontic irrigation and their combination in primary endodontic infections. *Int. J. Oral Health Sci.,* 10(2), 94.

17. Kamble, A. B., Abraham, S., Kakde, D. D., Shashidhar, C., & Mehta, D. L. (2017). Scanning electron microscopic evaluation of efficacy of 17% ethylenediaminetetraacetic acid and chitosan for smear layer removal with ultrasonics: an in vitro study. *Contemp. Clin. Dent,* 8(4), 621.

18. Eren, S. K., Uzunoğlu, E., Sezer, B., Yılmaz, Z., & Boyacı, İ. H. (2018). Mineral content analysis of root canal dentin using laser-induced breakdown spectroscopy. *Restor Dent Endod.* 43(1).

19. Teves A, Blanco D, Casaretto M, Torres J, Alvarado D, Jaramillo DE (2019). Effectiveness of different disinfection techniques of the root canal in the elimination of a multi-species biofilm. *J. Clin. Exp. Dent*; 11(11): e978–e983.

20. Segura-Egea, J., Gould, K., Hakan Şen, B., Jonasson, P., Cotti, E., Mazzoni, A., Sunay, H., Tjäderhane, L. & Dummer, P. (2018). European Society of Endodontology position statement: the use of antibiotics in endodontics. *Int. Endod. J.*, 51, 20-25.

21. Alshanta, O. A. M. (2022). Candida albicans and Enterococcus faecalis interkingdom interaction: implications for management of endodontic infections (Doctoral dissertation, University of Glasgow).

22. Asnaashari, M., Mojahedi, S. M., Asadi, Z., Azari

-Marhabi, S., & Maleki, A. (2016). A comparison of the antibacterial activity of the two methods of photodynamic therapy (using diode laser 810 nm and LED lamp 630 nm) against Enterococcus faecalis in extracted human anterior teeth. *Photodiagnosis and photodynamic therapy,* 13, 233-237.

23. Bibova, N., Simeonova, E., Dimitrova, S., Dakov, K., Denkova, Z., & Yanakieva, V. (2019). Photoactivated Disinfection of Root Canal System with FotoSan. *IOSR-JDMS.* 2279-0861. 18(9). PP 81-84.
24. Pandey, D., Paliwal, A., Singh, P., Brahmachary, B., & Singh, P. (2022). Antimicrobial Efficacy of Three Different Disinfecting Systems against E. Faecalis A comparative-in vitro study. *JPSP*. 6(2), 5415-5421.
25. Balakrishna, N., Moogi, P., Kumar, G. V., Prashanth, B. R., Shetty, N. K., & Rao, K. R. (2017). Effect of conventional irrigation and photoactivated disinfection on Enterococcus faecalis in root canals: An in vitro study. *JCD*, 20 (2), 125.
26. Nunes, M. R., Mello, I., Franco, G. C. N., de Medeiros, J. M. F., Dos Santos, S. S. F., Habitante, S. M., ... & Raldi, D. P. (2011). Effectiveness of photodynamic therapy against Enterococcus faecalis, with and without the use of an intracanal optical fiber: an in vitro study. *Photomedicine and laser surgery*, 29(12), 803-808.
27. Jambagi, N., Kore, P., Dhaded, N. S., Patil, S. A., & Shankar, M. (2021). Comparison of Antimicrobial Efficacy of Diode Laser, Ultrasonic Activated and Conventional Irrigation with 2.5% NaOCl during RCT: An Interventional Study. *JDCP*. 22(6), 669-673.
28. Rajakumaran, A., & Ganesh, A. (2019). Comparative evaluation of depth of penetration of root canal irrigant after using manual, passive ultrasonic, and diode laser–assisted irrigant activation technique. *J Pharm Bioallied Sci,* 11(Suppl 2), S216.
29. De-Deus, G., Belladonna, F. G., Simões-Carvalho, M., Cavalcante, D. M., Ramalho, C. N. M. J., Souza, E. M., ... & Silva, E. J. N. L. (2019). Shaping efficiency as a function of time of a new heat-treated instrument. *Int. Endod. J.* 52(3), 337-342.
30. Marchesan, M. A., Brugnera-Junior, A., Souza-Gabriel, A. E., Correa-Silva, S. R., and Sousa-Neto, M. D. (2018). Ultrastructural analysis of root canal dentine irradiated with 980-nm diode laser energy at different parameters. *Photomed Laser Surg*.26(3), 235-240.
31. Plotino, G., Grande, N. M., & Mercade, M. (2019). Photodynamic therapy in endodontics. *Int. Endod. J.*52(6), 760-774.
32. Carrera, E. T., Dias, H. B., Corbi, S. C. T., Marcantonio, R. A. C., Bernardi, A. C. A., Bagnato, V. S., ... & Rastelli, A. N. D. S. (2016). The application of antimicrobial photodynamic therapy (aPDT) in dentistry: a critical review. *Laser physics*, 26(12), 123001.
33. Ghinzelli, G. C., Souza, M. A., Cecchin, D., Farina, A. P., & de Figueiredo, J. A. P. (2014). Influence of ultrasonic activation on photodynamic therapy over root canal system infected with Enterococcus faecalis–an in vitro study. Photodiagnosis and Photodynamic Therapy, 11(4), 472-478.