



In vitro study the effect of laser photon-induced photoacoustic streaming on the enterococcus faecalis biofilm in complicated root canal system

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Article history: Received 11 Sept.2023; Revised 26 Nov. 2023; Accepted 2 Dec. 2023; Published online 15 Jun 2024

Abstract

Objective: The purpose of this in vitro study was to assess the efficacy of an erbium, chromium: yttrium scandium gallium garnet (Er, Cr: YSGG) laser using photon-induced photoacoustic streaming (PIPS) in the agitation of irrigation fluids in the complicated root system that infected with *Enterococcus faecalis* (*E. faecalis*).

Methods: The mesial roots of 90 recently extracted first and second lower human molars were separated, injected with *Enterococcus faecalis* suspension (except for the negative control group samples), and cultivated for thirty days. The samples were divided into five groups (n=15), first group acted as a positive control (inoculated untreated) (G1), syringe irrigation groups were irrigated with 5.25% sodium hypochlorite (NaOCl) (G2) and 2% chlorhexidine gluconate (CHX) (G4), laser groups were irrigated by 5.25% NaOCl (G3) and 2% CHX (G5) with Er, Cr: YSGG laser activation at 700 μ s, 5 Hz, (0.25, 0.5, 0.75, 1, and 1.25) W. AFM, or atomic force microscope, was employed as a novel technique to obtain data in the isthmus region. A scanning electron microscope (SEM) was also used in the study to confirm the results obtained from an atomic force microscope parameter. Statistical Package for Social Sciences (SPSS) software was used to collect and analyze data, and the study groups' means were compared using analysis of variance (ANOVA).

Results: After the results were statistically analyzed, the laser group with 2% chlorhexidine gluconate and 5.25% sodium hypochlorite showed a significant decrease in surface roughness than the syringe irrigation and the positive control groups ($p < 0.05$).

Conclusions: Based on the investigation's findings, the agitation of 2% chlorhexidine gluconate solution by Er, Cr: YSGG laser in photon-induced photoacoustic streaming at 1 W offers a better mechanism for bacterial biofilm removal than the conventional treatment technique. Whereas activation of 5.25% sodium hypochlorite with low-power laser at 0.25 and 0.5 W increased its efficacy.

Keywords: Atomic force microscope, 2% chlorhexidine gluconate, *Enterococcus faecalis* biofilm, Er; Cr: YSGG laser, sodium hypochlorite.

1. Introduction

The most well-known cause of root canal therapy failure is the continuation of intra-radicular infection due to inadequate bacterial clearance (Dioguardi et al., 2019). Microorganisms may survive even in fully managed teeth in dentinal tubules, canal irregularities, deltas, and isthmus regions (Nair, 2004). The isthmus



is a communication between two or three root canals with pulp tissue within them. Its anatomic complexity results in challenging debridement. The prevalence of isthmus in the mesial roots of permanent lower molars ranges from 80.6% to 100% on complete root investigation (Natanasabapathy et al., 2021). The purpose of the irrigating solutions is to clean and disinfect the portions of the root canal system that have skipped instrumentation because more than half of the root canal walls have remained untouched after canal instrumentation (Peters et al., 2001).

The most commonly used antibacterial irrigation solutions are sodium hypochlorite (NaOCl), which is an efficient organic solvent as well as a powerful antibacterial agent (Cullen et al., 2015), and chlorhexidine gluconate (CHX), which is a broad-spectrum antibacterial fluid that works to combat both gram-positive and gram-negative bacteria as well as yeasts (Abdelgawad et al., 2020). However, the isthmus regions are not accessed by instruments and may not be reached by irrigation solutions due to restricted penetration and diffusion of the irrigation solution, so the microorganisms may persist in these regions (Berutti et al., 1997). As a result, it is critical to work on novel strategies to ensure irrigation fluid reaches inaccessible places, thereby improving endodontic outcomes. Recently, the laser photon-induced photoacoustic streaming (PIPS) technique was introduced to activate the irrigation solution for improving root canal disinfection and cleaning (Rasheed and Jawad, 2021). This technique is used by an Erbium laser family in sub-ablative settings. An erbium, chromium: yttrium scandium gallium garnet (Er, Cr: YSGG), is a type of water-absorbing laser widely used in endodontics (Al-Karadaghi et al., 2015). Many factors are known to influence PIPS effectiveness, including the irrigant used, the canal dimensions, the area to be disinfected, and laser settings (Zhu et al., 2013). In this investigation, atomic force microscopy (AFM) was used to evaluate biofilm removal, which is a computer-driven mechanical microscope that can report surface roughness values quantitatively and provide high-resolution three-dimensional topographical imaging of biological samples.

This method has been commonly used to examine the mechanism of antimicrobial activity on bacteria, and it is a helpful instrument for biologists (Gadegaard, 2006). López-Jiménez et al. used AFM to examine the changes on the surface of *E. faecalis* after treatment with Erbium laser and diode lasers and concluded that AFM is a useful tool for determining microbial survivability as a measure of antimicrobial efficacy (López-Jiménez et al., 2015). While Kumar et al. assessed the efficiency of laser activation of sodium hypochlorite in eliminating multispecies biofilms from the mesial root of permanent molars using confocal microscopic imaging and found that the laser agitation displayed effective isthmus cleaning (Kumar et al., 2022).

The goal of this investigation was to assess the efficacy of Er, Cr: YSGG laser in PIPS by agitation of irrigation fluids in the isthmus, which was infected with a mature bacterial biofilm.

2. Materials and Methods

The Helsinki Declaration was considered in this study. The procedures utilized in this investigation obtained ethical approval. (BU-2022-488).

2.1 Selection and preparation of samples

90 recently extracted first and second lower human molars with an isthmus in the mesial root were chosen from a group of 100 molars (patients aged 23 to 40 years old). The teeth were collected from November 2022 to June 2023 at Martyr Dr. Wassim Dental Specialist Center/ Department of Oral Surgery. The reason for extraction was pulp pathology. All collected molars were imaged using cone-beam computed tomography and samples that have a continuous isthmus between the mesiobuccal and mesiolingual canals were selected (Figure 1) (Bago et al., 2023). Then all samples were kept in plastic tubes containing saline water and 0.1% crystals of thymol (Lab Grade, Lab Alley, Texas, USA) until the experiment' day.



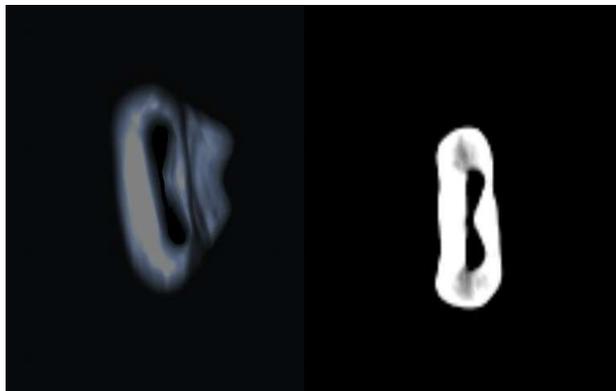


Fig.1. A CBCT image of a lower molar mesial root shows the isthmus between the mesiolingual and mesiobuccal canals.

The working length (WL) was determined by sighting the file tip through the apical foramen and subtracting 1 mm from the observed file length (Kumar et al., 2022). All canals were instrumented to this working length up to size 25/.04 NiTi engine files (X3 Never Break, Easy smile Co., USA) at speed (300 rpm) and torque (2 Ncm), as suggested by the manufacturer (Kimura et al., 2020). 5.25% NaOCl (Cerkamed, Poland) was delivered after each file size during the preparation process using an irrigation needle with side opening (Endo-Top, Hang Zhou Endo-Top Co., China). One ml of 17% EDTA (Cerkamed, Stalowa Wola, Poland) was utilized as the last irrigant, and it was kept inside for three minutes. During this time, the solution was stimulated for 30 seconds with an ultrasonic instrument (Guilin-Woodpecker Co., China).

The residual irrigation solutions were then removed by rinsing all of root samples with five milliliters of distilled water (Pioneer-Company, Iraq). cotton pads were used to clean the root's surface, and paper points were used to dry the canals (Sure endo, Sure-Dent Corporation, Korea) (Parente et al., 2010). All roots were separately inserted in Eppendorf tubes (Lab-Serv et al., India) and sterilized for 20 min at 121°C and 15 psi pressure.

2.2 Bacterial inoculation

E. faecalis colonies were obtained from an agar plate (Himedia, Mumbai, India), which was previously streaked by bacteria. Then activated by placing them in brain heart infusion (BHI) broth (Himedia, Mumbai, India) for 24 h. The obtained suspension was then subject to a series of dilutions to obtain suspension equal to the standard of McFarland (1.5×10^8 CFU/ml) (Cheng et al., 2012).

The bacterial suspension was introduced into the root canals (except for 15 roots, which act as a negative control) using a 30 gauge irrigation needle until the canals were filled completely (Cheng et al., 2016). After that, each root specimen was placed in an Eppendorf tube and submerged in 1.5 ml of BHI broth. All sample containers were maintained under aerobic conditions at 37°C for 30 days. To ensure the availability of living bacterial cells during the incubation period, re-inoculation of the canals with the suspension was done every three days. The BHI was also replaced every day with a fresh one to provide good nutrition.

2.3 Treatment groups

At the end of the incubation time, an injection needle was used to remove the liquid medium from the tubes, and each root sample was subjected to multiple processes, including cleaning the samples' surfaces with sterile cotton pads dipped in 5.25% NaOCl. The samples were then placed in plain tubes filled with impression material (Kro-malgin, Vannini-dental, Italy) for easier handling. In pilot study, the laser pulse repetition rate was examined with different values (5, 10, and 15 Hz) and 5.25% NaOCl used as an irrigant. The results were obtained by the traditional colony formation unit (CFU). Finally divided randomly into five groups:

G1: The positive control group (n=15)

The samples of this group did not get any sort of therapy.

G2: 5.25% NaOCl+ syringe irrigation (SI) group (n=15)

The sample's canals were irrigated with 1 ml of 5.25% NaOCl delivered by 30-gauge irrigation needle and kept inside the canals for 2 min, then washed with 5 ml of distilled water.

G3: 5.25% NaOCl + Er, Cr: YSGG laser group (n=15)

The sample's canals were irrigated with 5.25% NaOCl and kept inside the canals for 2 min, throughout this time, the fluid was activated by a 2780 nm Er, Cr: YSGG laser (Biolase, Waterlase, CA, USA) at 700 μ s, 5 Hz according to the pilot study, and (0.25, or 0.5, or 0.75, or 1, or 1.25) W for 60 seconds, three samples for each power. The laser was activated for 30 sec of (on) time, followed by 30 sec of (off) time, which was repeated twice (an overall duration of 60 sec). The canals were then rinsed with 5 ml of distilled water.

G4: 2% CHX +SI group (n=15)

An irrigation needle (30 gauge) was used to deliver 2% CHX irrigant in to the canals and kept inside the root canals for 2 min, then rinsed with 5 ml of distilled water.

G5: 2% CHX+ Er, Cr: YSGG laser group (n=15)

The sample's canals were irrigated with 2% CHX and kept inside the canals for 2 min, within this period, the irrigant was activated by a 2780 nm Er, Cr: YSGG laser for 60 sec at the same settings of the previous laser agitation group (G 3). The laser was activated for 30 sec of (on) time, followed by 30 sec of (off) time, which was repeated twice. The canals were then rinsed with 5 ml of distilled water. Prior to laser activation, infrared laser safety glasses (Innovative-Optics, Hemlock, USA) were worn. A new design water Lase iPlus /MD tip (MZ6 tip diameter = 600 μ m, length = 6 mm) was utilized.

The settings of water and air spray of the laser unit were both set to (off). The tip was placed just inside the canal opening, stayed fixed, and was not moved apically into the root canal during the procedure.

3. Preparation of the samples for AFM and SEM observation

In order to keep tooth-cutting debris out of the canals and isthmus, proper-sized paper points were put in the root canals. All roots were marked and cut longitudinally with a double-face diamond disk. A middle area of the isthmus area was marked for observation by the AFM (Nanosurf, Liestal, Switzerland) (Figure 2).

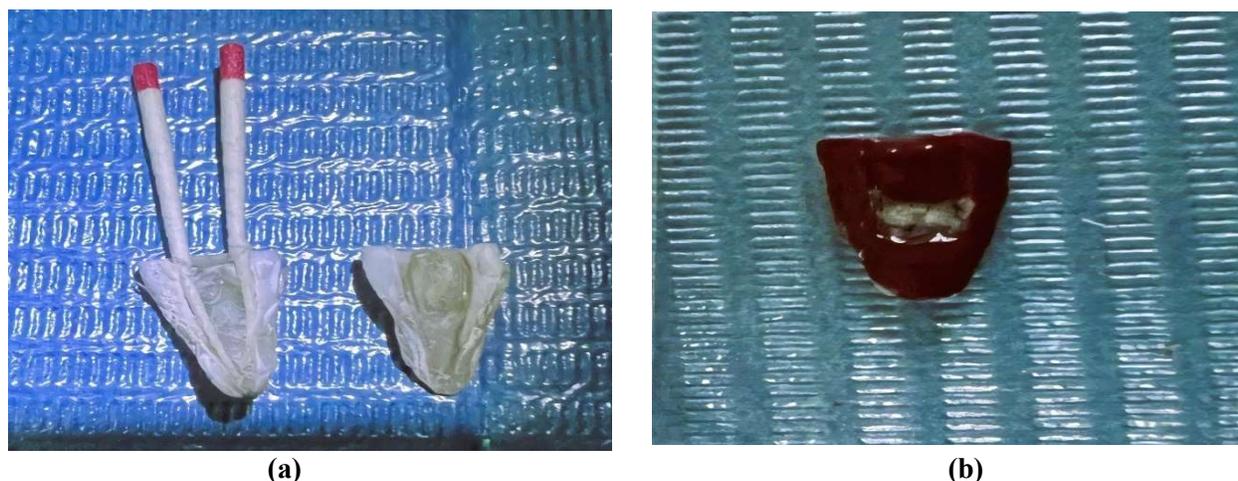


Fig. 2.Root sectioning for AFM inspection: (a) a longitudinally divided root sample; (b) the center portion of the isthmus is marked on one root division prepared for the AFM observation.

The remaining halves of the roots were mounted on an aluminum base and metalized with a gold covering by vacuum evaporation, then a scanning electron microscope (SEM) (Inspect F-50, FEI Electron Optics International B.V., Netherlands) was used to examine the samples at 13 000 magnification power.

4. Statistical analysis

The Statistical Package for Social Sciences (SPSS) version 21 (IBM, Armonk, USA) was used to analyze the data. It was presented as a mean, and categorical data was represented by the standard deviation. The means of the tests were compared using analysis of variance (ANOVA). The least significant difference (LSD) test was used for calculating the significant differences among the tested means. Letters (A, B, C, D, E, and F) demonstrated the levels of significance, the letter (A) being the most significant and decreasing with the last one. Identical letters indicate that there are no significant variances in the measured means. $p > 0.05$ results were regarded statistically not-significant, whilst $p \leq 0.05$ was considered a significant value.

5. Results

Relatively low peaks and shallow valleys can be seen on the exterior of the uninoculated isthmus surface in figure 3 three-dimensional (3D) AFM image. The infected isthmus surface was depicted in figure 4 using an AFM image that had high peaks and low valleys. Also, a three-dimensional image of the isthmus surface after treatment with 2% CHX agitated by laser PIPS at 1 W showed a bumpy surface with round peaks and a few shallow valleys (Figure 5).

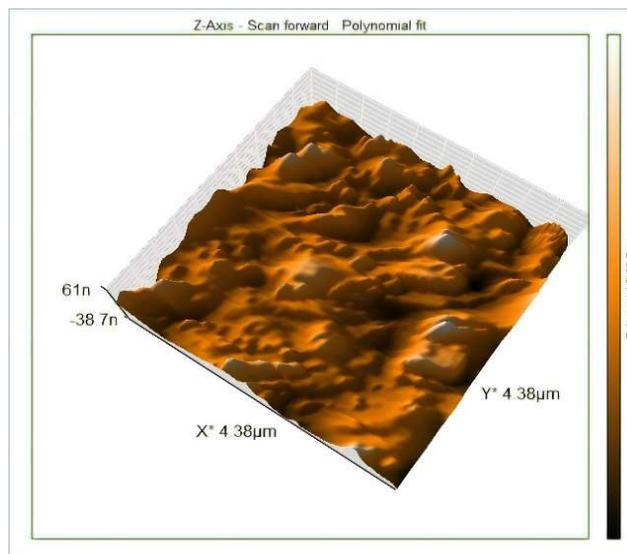


Fig.3. Three dimensions AFM image of uninoculated isthmus area about 6 mm away from the apex.

The root mean square roughness (Sq.) values that were measured for all groups samples were statistically analyzed and then compared to each other by ANOVA. The isthmus surface roughness was different among all groups under study ($p \leq 0.05$). The lowest root mean square value was presented in the 2% CHX group that agitated by laser PIPS at 1 W (G 5), followed by 5.25% NaOCl group that agitated by laser PIPS at (0.25 or 0.5) W (G 3), 5.25% NaOCl+ SI group (G 2), and 2% CHX + SI group (G 4) respectively. The highest root mean square value was presented in the positive control group (G1).

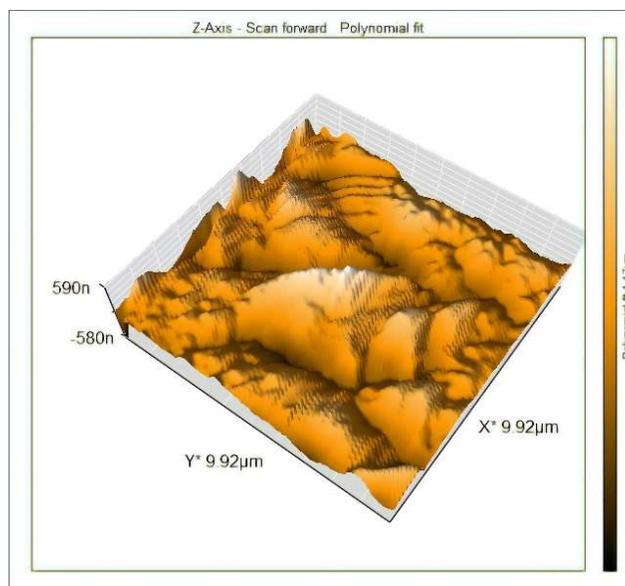


Fig. 4. Three dimensions image of the isthmus surface after inoculated with the bacteria suspension for 30 days.

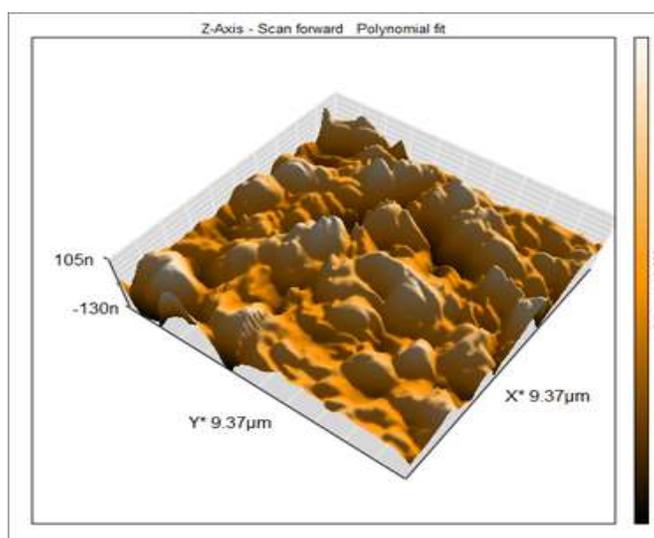


Fig.5. Three dimensions image of the isthmus surface after treatment with 2% CHX agitated by laser PIPS at 1 W.

There was no significant difference found between the laser agitated at (0.25 or 0.5) W with NaOCl in this regard. The results of all test groups are shown in tables 1, 2, and 3 and in Figures 6 and 7.

Before the treatment, field emission scanning electron microscopy (FE-SEM) pictures were obtained at a distance of roughly 6 mm from the apex. These images showed that the isthmus surface was covered with debris and a thick layer of biofilm (Figure 8 a). On the other hand, after the CHX and laser treatment at 1 W SEM images showed a clean surface with open dentinal tubules (Figure 8b).

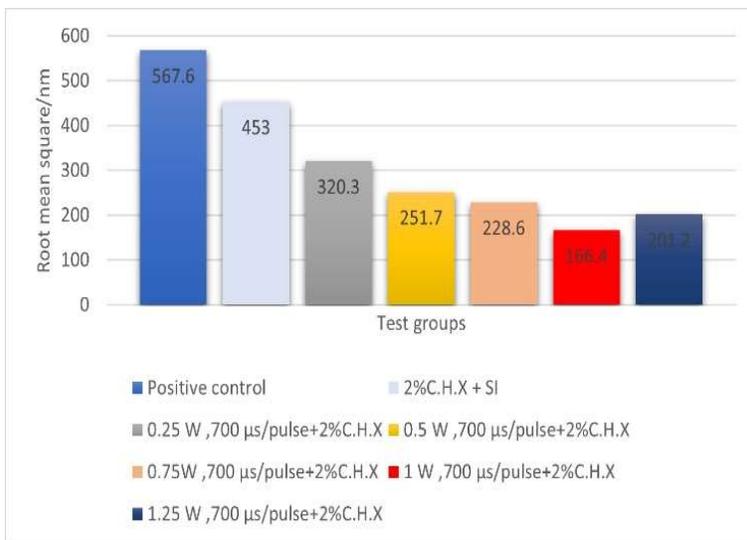


Fig. 6. Statistical columns chart showing the means of root mean square roughness values of positive control group (G 1), 2% CHX + needle irrigation group (G 4), and laser agitation 2% CHX group (G 5), the red column represents the best result.

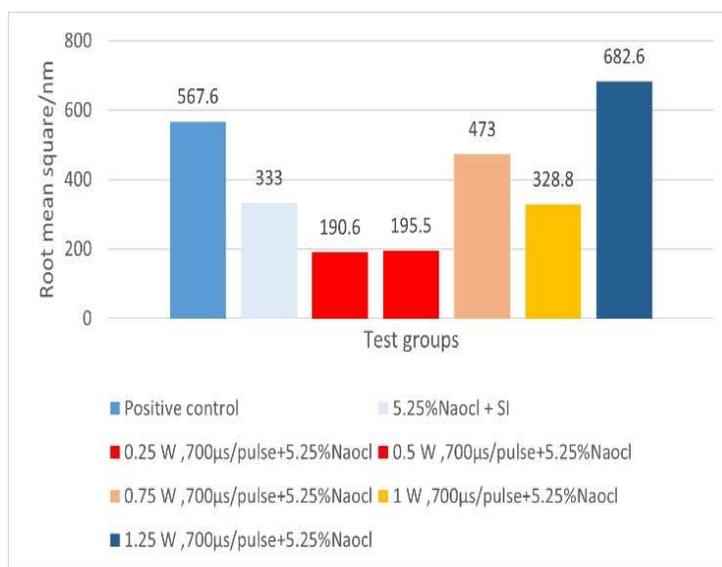


Fig.7. Statistical columns chart showing the means of root mean square values of positive control group (G 1), 5.25% NaOCl + needle irrigation group (G 2), and laser agitation of 5.25% NaOCl group (G 3), the red columns represent the best result.

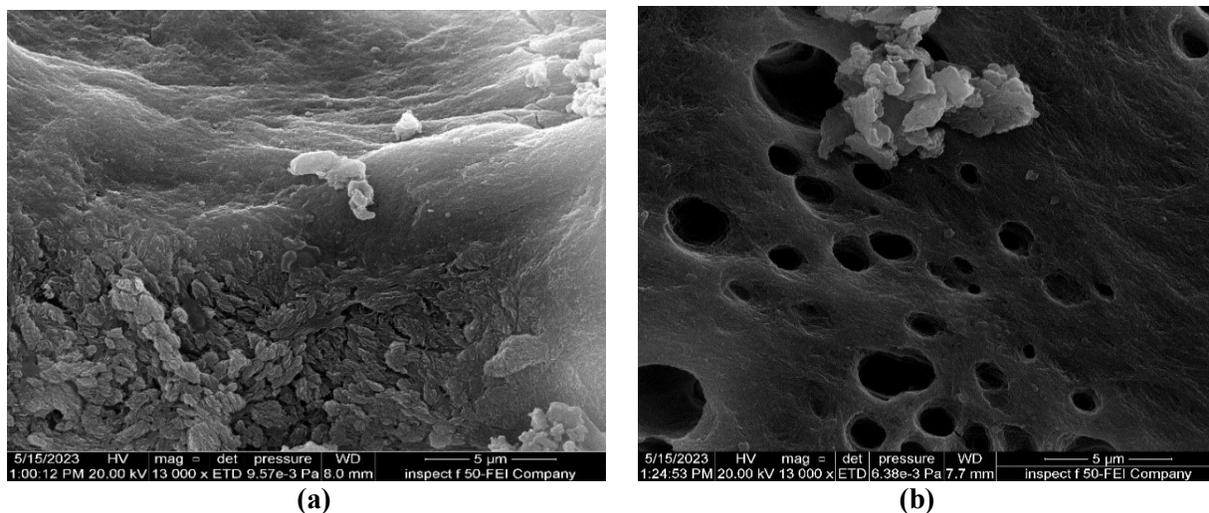


Fig. 8. FE-SEM 13 000 magnification of the isthmus about 6 mm away from the apex (a) A positive control specimen (b) The same isthmus area after irrigated with 2% CHX + PIPS at 1 W.

Table 1. Means and standard deviations of root mean square values obtained from all samples treated with 5.25% NaOCl, means compared by one-way and two-way ANOVA.

Tested groups /5.25% NaOCl	Sq (root mean square) nm	P value
Negative control	17.73±3.9	
Positive control (G 1)	D 567.6±26.2	0.001 HSIG
5.25% NaOCl + SI (G 2)	B 333.0±22.1	
Laser group + 5.25% NaOCl (G 3)		
0.25 W, 700 μs	A 190.6±8.9	0.001 HSIG
0.5 W, 700 μs	A 195.5±14.2	
0.75 W, 700 μs	C 473±27.8	
1 W, 700 μs	B 328.8±12	
1.25 W, 700 μs	E 682.6±16.2	
P value	0.001	

Abbreviations: Sq.: Root mean square roughness nm: Nanometer NaOCl: Sodium hypochlorite SI: Syringe irrigation W: Watt (unit of power), μs: Microsecond HSIG: Highly significant P value: Probability value

Table 2. Means and standard deviations of root mean square values obtained from all samples treated with 2% CHX, means compared by one-way and two-way ANOVA.

Tested groups /2% CHX	Sq (root mean square) Nm	P value
Negative control	17.73±3.9	
Positive control (G 1)	F 567.6±26.2	0.001 HSIG
2% CHX + SI (G 4)	E 453.0±34.1	
Laser group + 2% CHX (G 5)		
0.25 W, 700 µs	D 320.3±14.8	
0.5 W, 700 µs	C 251.7±28.5	0.001 HSIG
0.75 W, 700 µs	B 228.6±26.3	
1 W, 700 µs	A 166.4±11.6	
1.25 W, 700 µs	B 201.2±12.9	
P value	0.001	

Abbreviations: Sq.: Root mean square nm; Nanometer CHX: Chlorohexidine gluconate SI: Syringe irrigation W: Watt (unit of power), µs: Microsecond HSIG: Highly significant P value: Probability value

Table 3. Means and standard deviations of root mean square values obtained from all samples treated with 2% CHX and 5.25% Naocl with laser agitation, means compared by one-way ANOVA.

Laser groups / 700 µs/pulse / Sq. (root mean square) nm			
Tested groups	2% CHX 700 µs/pulse (G 5)	5.25% Naocl 700 µs/pulse (G 3)	P value
0.25 W	320.3±14.8	**190.6±8.9	0.001
0.5 W	*251.7±28.5	**195.5±14.2	
0.75 W	*228.6±26.3	473±27.8	
1 W	***166.4±11.6	328.8±12	
1.25 W	*201.2±12.9	682.6±16.2	

***, **, *** Significant groups**



6. Discussion

Isthmuses act as a considerable challenge area for root canal cleaning and obturation; their ribbon shape, size, and extension laterally from the main canal make mechanical instrumentation impossible (Robberecht et al., 2023). So, irrigant solutions used during root canal preparation should be able to penetrate the entire root canal system for better endodontic outcomes and prognosis (Kumar et al., 2022). The current study showed how the efficiency of removing the biofilms from isthmuses was influenced by the laser agitation mechanism. In many past studies, molecular and culture methods have been used to determine the number of live bacteria in the root canals (Hoedke et al., 2021). Additionally, the viability of microorganisms in the lateral canals, isthmus, and root canal walls can be determined using a confocal laser microscope (CLSM) (Kumar et al., 2022). An atomic force microscopy (AFM) tool was used to examine the samples because it is easy to apply, precise, and available. The topography of the surface of the isthmus was studied by analyzing of surface roughness and particle size. Surface roughness analysis was characterized by calculating the height parameters including maximum height parameter (Sz.), root mean square roughness parameter (Sq.), and average roughness parameter (Sa.). In the present investigation, (sq.) was the dependent variable, which is regarded to be more sensitive to considerable deviations from the mean line than average roughness (Kumar and Rao, 2012).

According to the data, the isthmus surface that was not infected with bacteria had a computed root mean square value of extremely low (17.73 ± 3.9 nm), whereas the surface after mature biofilm formation had a significantly higher sq. value of around (567.6 ± 26.2 nm) ($p < 0.05$). The surface's increased roughness indicates that the biofilm growth produced a rougher and more irregular surface. The measured sq. value of the samples that were treated with 2% CHX and Er, Cr: YSGG laser at ($700 \mu\text{s/pulse}$, 5 Hz, 1 W) of about (166.4 ± 11.6 nm) is significantly less than the values measured after other methods ($p < 0.05$). This indicates that laser PIPS agitation was a more successful method than other conventional techniques in minimizing the amount of *E. faecalis* biofilm on the isthmus surface.

PIPS is an advanced laser-agitation irrigation technology in which laser photons are generated at low energy levels and with short microsecond pulse durations. This enables lateral propagation of the shock wave in fluids at sub-ablative levels via photoacoustic and photomechanical phenomena. This eliminates the risk of thermal damage and enables effective three-dimensional streaming (Olivi and DiVito, 2016).

The laser PIPS technique is achieved by absorbing the laser energy by the irrigation fluid that fills the root canals, and without this absorption, no laser action is achieved. Therefore, the wavelength of the used laser must be near to the peak of the strong absorption of the material (Frayssinous et al., 2018). 2780 nm Er, Cr: YSGG laser are well-absorbed by water chromophore (Rand kareem Jassim, 2022). So high absorption of this laser energy is ensured by the irrigation solution used in this study, and a strong pressure and shockwaves propagate three-dimensionally within the root canal systems that are filled with fluids (Olivi and DiVito, 2016). Therefore, the irrigants can reach the difficult-to-access areas without needing to put the tip near the morphologically thinning apical third.

As it is known, the sodium hypochlorite irrigant is effective in the removal of bacterial biofilm, so a low-power laser was enough to improve its efficacy (Al Shahrani et al., 2014), which reflected in a decrease in the surface roughness at 0.25 and 0.5 W about of (190.6 ± 8.9 and 195.5 ± 14.2 nm). While, the CHX irrigation solution has low efficacy in removing biofilm (Clegg et al., 2006). So higher power is required not only to pump the liquid by generating a shock wave but also to create stronger cavitation, which makes the bacterial cell membrane weaker and easier for irrigants to enter (Gu et al., 2009). However, the resulting photomechanical phenomena worked to increase the effectiveness of irrigation solutions in disrupting the bacterial biofilm. The irrigation syringe becomes more efficient in conventional treatment when the needle end gets closer to the working length (Boutsoukis and van der Sluis, 2015). While, in laser PIPS agitation, the tip of laser device is not placed inside the canal and is restricted to coronal access in the canal orifice. Therefore, the PIPS technique allows minimum root canal preparation in order to retain healthy tooth structure, according to the minimally invasive endodontics (MIE) approach (Anjum et al., 2019) while ensuring that the irrigants can reach hard-to-reach areas. The SEM images revealed a large part of the isthmus surface and dentinal tubules as being free of bacterial biofilm after treatment with the laser agitation



method. This confirms the results obtained from AFM analysis and proves that the laser agitation is very effective in removing the biofilm from these areas.

The present study results are in a good agreement with many studies that tested the antimicrobial effect of Er family lasers and obtained similar results. Sahar-Helft et al. (Sahar-Helft et al., 2013), examined the actions of 2% CHX and Er: YAG using LAI on *Enterococcus faecalis* biofilm reported that the total number of microorganisms dramatically decreased after being treated with LAI and CHX. While, Aydin et al. (Aydin et al., 2020), compared the antimicrobial effects of sodium hypochlorite and 2% CHX irrigants agitated by Er, Cr: YSGG laser (LAI), and discovered that activating NaOCl and CHX irrigation fluids with Er, Cr: YSGG pulsed laser can be effective in the eradication of *E. faecalis* bacteria from the canals. This experiment was limited by the possibility of tiny fragments forming during the cutting stage, which could raise the surface roughness rating. So, a paper point was inserted into the canal to keep debris out of the isthmus during the cutting process, however, this helped to lessen this disadvantage. Further limitation that could impact the results is the anatomical variance in the isthmus area of the chosen teeth.

7. Conclusions

In conclusion, the data given in this study indicate that Er, Cr: YSGG laser agitation improve the removal of microbial biofilm more than standard irrigation methods delivered by irrigation syringes. Furthermore, this laser had improved the effect of 2% CHX at a power value of 1 W, while agitation of 5.25% NaOCl by low-power laser at (0.25 and 0.5 W) was improved the sodium hypochlorite efficacy against the mature biofilm.

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دراسة مختبرية لتأثير التدفق الصوتي المستحث بالفوتون بالليزر على الأغشية الحيوية للمكورات المعوية البرازية في نظام قناة الجذر المعقد

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الخلاصة

الهدف: الغرض من هذه الدراسة المختبرية هو تقييم فعالية ليزر الإربيوم والكروم: الإيتريوم سكانيديوم الغاليوم باستخدام التدفق الصوتي الصوتي المستحث بالفوتون في تنشيط سوائل الري من خلال تقييم تأثيره في اإبادة البكتريا في نظام الجذر المعقد الذي تم استعماره بالمكورات المعوية البرازية.

الطرق: تم فصل الجذور الاونسية لـ 90 ضرساً سفلياً مقلوع حديثاً، وحقتها بمعلق المكورات المعوية البرازية (ما عدا عينات المجموعة السيطرة السلبية)، وحضنت لمدة 30 يوماً. تم تقسيم العينات إلى خمس مجموعات (العدد = 15)، المجموعة الأولى كانت بمثابة السيطرة الإيجابية (الملقحة غير المعالجة) (G1)، تم ري مجموعات الري بالمحاقن باستخدام هيبوكلوريت الصوديوم 5.25% (G2) و2% جلوكونات الكلوروهيكسيدين (G4) ، وري مجموعات الليزر بهيبوكلوريت الصوديوم 5.25% (G3) و2% جلوكونات الكلوروهيكسيدين (G5) التي تم تنشيطها بالليزر عند 700 ميكروثانية، 5 هرتز، (0.25، 0.5، 0.75، 1، و1.25) واط. تم استخدام مجهر القوة الذرية كطريقة جديدة للحصول على النتائج في منطقة البرزخ؛ تم أيضاً استخدام المجهر الإلكتروني الماسح في الدراسة لتأكيد النتائج التي تم الحصول عليها من مجهر القوة الذرية. وايضا تم استخدام برنامج الحزمة الإحصائية للعلوم الاجتماعية لجمع وتحليل البيانات، واستخدم تحليل التباين لمقارنة مجموعات الاختبار.

النتائج: بعد تحليل النتائج إحصائياً، أظهرت مجموعة الليزر التي تحتوي على 2% جلوكونات الكلوروهيكسيدين و5.25% هيبوكلوريت الصوديوم انخفاضاً ملحوظاً في خشونة السطح مقارنة بالري بالمحاقن التقليدية ومجموعة السيطرة الإيجابية.

الاستنتاجات: استناداً إلى نتائج الدراسة، فإن تنشيط محلول 2% جلوكونات الكلوروهيكسيدين بواسطة الليزر باستخدام تقنية التدفق الصوتي الصوتي الناجم عن الفوتون عند 1 واط يوفر إزالة أفضل للأغشية الحيوية البكتيرية الناضجة من تقنية المعالجة التقليدية. بينما أدى تنشيط هيبوكلوريت الصوديوم باستخدام الليزر منخفض الطاقة عند 0.25 او 0.5 واط أدى إلى زيادة فعاليته.

