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Cavity Disinfection Using Er,Cr:YSGG Laser Induced Photoacoustic Streaming Technique

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Abstract:

Aim: The goal of this research was to study the influence of Er,Cr:YSGG laser at short pulse duration (60 µsec) on the number of streptococcus mutans bacteria in vitro.

Material and Methods: twenty-eight extracted third molars free of caries, cracks, and other irregularities were used. For the testing of the materials, both the agar well technique and a tooth cavity model were employed. The agar wells of plates that had been inoculated with Streptococcus mutans previously were stuffed with the test materials, in order to conduct the tests. The zones of inhibition were assessed using millimeter measurements, after an incubation period of 48 hours .In order to accomplish the tooth cavity model test, cylindrical cavities were invented in the occlusal surface of the teeth, which was kept even. The teeth were stored for 72 hours at 37°C in a broth culture of Streptococcus mutans. Following this, the teeth were divided arbitrarily into four groups of seven teeth (each including 14 cavity preparations). The experimental cavities in the first group (A) were not treated and considered as a control. In group B, a cavity disinfectant based on chlorhexidine was applied to the experimental cavities for 60 seconds. In group C, an erbium, chromium doped yttrium scandium gallium garnet laser was used at a short pulse duration (60 µsec) (0.25 watts, 15 Hz, 1% air, 1% water). In the last group, a chlorhexidine cavity disinfectant was applied for 60 seconds, followed by a laser treatment for 30 seconds with the same parameters as those described previously. The teeth were stored in saline for a period of three days. Standard amounts of dentin chips were retrieved from the cavity walls. ANOVA test was used to analyze repeated measure mean between tested concentration and control. Data expressed as mean ±SE. LSD tests was used to calculate the significant differences between tested mean.

Result: After the statistical test, the highly significant difference in the diameter of inhibition zone was observed in group D (26 mm) where both chlorhexidine and laser were used followed by group B (18.71 mm) where the chlorhexidine gluconate based cavity disinfectant used alone, the least significant difference observed in group C (10.26mm) where the laser used alone.

Conclusion: According to this in vitro study, a photon-induced photoacoustic streaming technique using an Er,Cr:YSGG pulse laser at short pulse duration effectively agitates a chlorhexidine-based cavity disinfectant, which leads to the inhibition of Sterptococcus Mutans.

Keywords: Er,Cr:YSGG ,PIPS, Strep.Mutans, Inhibition.

1. Introduction

The primary goal of caries removal is to eliminate the diseased and necrotic tissues as well as any bacteria that may have the potential to cause persistent inflammation and treatment failure. Therefore, the total removal of the diseased dentin has a direct influence and impact on the clinical success of a restoration. However, the caries treatment techniques that are operated at the present time do not always or absolutely eradicate all of the microbes that are present in residual tissues) .Boston and Graver ,1994)

Effort at completely remove deep carious dentin using only mechanical techniques, may lead to risk of damaging the pulp of the tooth and/or causing extensive tooth structure loss.(de Almeida *et al*, 2011)

The strategy of caries removal using only mechanical methods are unsuccessful in producing a cavity that is absolutely free of caries (Cheng *et al*, 2013).Subsequently, coating the cavity preparation with antibacterial chemicals to aid in the eradication of microorganisms began to gain widespread favor among dental practitioners. (Al-Omari *et al*, 2006) The chemo mechanical caries removal system regarded as a substitute to the conventional caries removal with round bur in a slow speed hand piece. It is effective and comfortable, even it requires a longer time. (Ismail and Haidar, 2019)

In clinical dentistry, multiple disinfectants have been employed in an effort to lessen or eliminate microorganisms during cavity preparation and before to the insertion of dental restorations. Because of their inherent chemicals, some of these agents, has been noted to cause pulpal irritation. As a result, their use has been ignored (Shafiei and Memarpour, 2012)

The continuous search for more effective techniques of dental treatment has led to the investigation of a wide variety of lasers, and it has been shown that the capabilities of lasers in the field of dentistry are indeed advancing. The removal of dental hard tissue by laser has become both safer and more effective when used in conjunction with a water spray, and as a result, this technique is acquiring a larger amount of appreciation in the field of restorative dentistry. Erbium lasers, in particular, have the capability of removing enamel, dentin, and carious tissue with clear enamel-dentin boundaries and clean surfaces of enamel and dentin with different micro-morphologies. The morphology of lased dental surfaces presented homogeneous alterations. (Chowdhury *et al*, 2017)

In comparison to any other laser that is applied for dental purposes, the Er:YAG laser light has the highest absorption rate in water, and its wavelength matches well with the absorption maximum of hydroxyapatite.(DiVito *et al*, 2012)

A highly absorbed laser produces reactive oxygen species that damage the bacterial membrane, resulting in the rapid death of microorganisms. (Yao *et al*, 2012)

Laser energy has the potential to not only eliminate bacteria directly but also to stimulate the irrigant, thereby augmenting the bactericidal effects of the irrigant. (de Groot *et al* ,2009; Meire *et al* ,2009)

The Er:YAG laser triggered in a restricted volume of fluid, the high peak power derived from the short pulse duration combined with its high wavelength absorption in water , resulted in a photomechanical phenomenon (DiVito *et al*, 2012)). The term "photon induced photoacoustic streaming" is used to describe this phenomena (PIPS). (Sabreen and Hussien, 2021)

PIPS a new laser activated irrigation (LAI**)** process using a very low power source (subablative), pulses the laser light energy which absorbed by the irrigant molecules. This energy transfer generates a sequence of fast and violent shock waves able to pushing the irrigant through the entire root canal structure with great force. (DiVito *et al*, 2012)

Due to the fact that the bactericidal impact of pulsed Er:YAG laser is non-thermal, it is possible to avoid the unfavorable consequences of thermal energy. (Guidotti *et al*, 2012)

The purpose of the present study was to examine the bactericidal effect of erbium and chromium: dopped yttrium-scandium-gallium garnet (Er,Cr:YSGG) at short pulse duration (60 sec) using photon-induced photoacoustic streaming (PIPS).

2. Material and Methods

Antibacterial activity of test materials was estimated using Streptococcus mutans, provided by the Department of basic science, University of Baghdad. The techniques used in the research were the agar well technique and the tooth cavity model.

Agar well technique- Colombia blood agare was evenly distributed over the surface of 15 cm. in diameter petri dishes to a thickness of 5 mm. 0.5 ml. of *Streptococcus mutans* suspension (10^6 cfu/ml) was inoculated by a bent glass rod over the agar surfaces. Wells with a diameter of 4 mm were then punched into the agar using the blunt end of a sterile Pasteur pipette. The plates were then divided into three groups, each with seven plates. The well in the first group (I) loaded with 2% Chlorohexdine gluconate-based cavity disinfectant alone , in the second group (II) the well irradiated by erbium, chromium, yttrium, scandium, and gallium garnet laser (25 W, 15 Hz, 1% air, 1% water) using photon-induced photoacoustic streaming with short pulse duration (60 sec) .Lastly, chlorohexidine gluconate (2%CHX) was applied in the wells of the third group (III) then exposed to Er,Cr:YSGG laser radiation using the same setting stated above . Then the plate incubated at 37 C for 24 h. A sliding caliper was used to measure the diameter of the inhibition zone surrounding the wells in two randomly selected areas. The zone of inhibition (ZOI: mm) that appeared around the wells was recorded. *Tooth cavity model technique* - This part of the study was accomplished according to the manner used by Özer *et al* (Özer *et al, 2003*).

In order to complete this process, 28 human molars that were completely free of caries, restorations, and other flaws were used. In order to create flat dentin surfaces, the enamel of the teeth was amended horizontally with a water-cooled diamond bur to gain even dentin surfaces. Two cylindrical cavities 1mm in diameter, 2 mm depth) were prepared on the flat surface of each tooth without exposing the pulp as show in (Fig. 1).

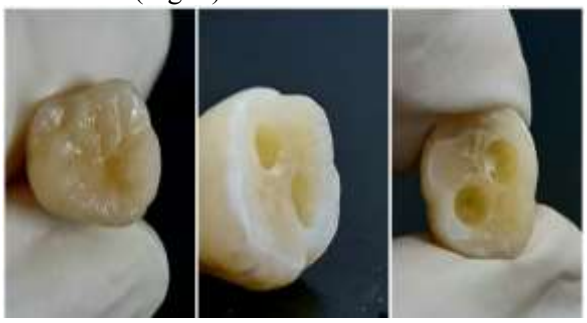


Figure (1): Preparation of the control and experimental cavities

The teeth were divided at random into four groups containing seven teeth (14 cavity preparations) each. The teeth were sterilized by autoclave for 15 minutes at a temperature of 121 ℃. The teeth kept in brain heart infusion (BHI) broth and incubated for 24 hours at 37°C, to confirm sterility. For washing out the culture medium and to avoid dehydration, each tooth was transferred in an individual tube hold 2 ml of sterile physiologic saline (SPS) and stored for 24 hours at 37°C. After that the teeth desiccated with sterile paper point and a gentle stream of air. To establish infected cavities, all the teeth were placed in a bottle containing broth culture of *Streptococcus mutans* suspension (10^6 CFU/ml) and incubated at 37°C for 72 hours. In group A the cavities were left untreated and served as the control. For Group B, the CHX 2% was applied into the cavities using a sterile brush, left undisturbed for one minute, and then it was gently dried with an air syringe. The experimental cavities in Group C treated using Er,Cr:YSGG laser (Waterlase, Biolase, California, USA) with 0.25 W , 1% water jet, and 1% air jet for a period of 30 seconds. In Group D, both 2% CHX based cavity disinfectant and Er,Cr:YSGG laser with the same parameter used above were applied to the experimental cavities.

Following the application of a temporary restorative material (Cavit GC) to the occlusal surfaces of the teeth, each tooth was placed in its own container of sterile physiological saline and stored at 37 ˚C for 72 hours. The teeth were then removed from the SPS, and standardized amounts of dentin chips (20 ± 5 mg) were collected from the cavity using a new sterile steel bur, mounted to a low-speed contra-angle hand piece, and then placed into sterile tubes. For every cavity, a new sterile bur was used to avoid overheating of dentinal walls during the cutting process. Adding 2 ml of sterile physiological saline into the suspensions with the dentin chips and mixed using Vortex for 30 seconds to enable the microorganisms to pass through the solution, thus produce a consistent suspension. Serial dilutions of 10−1, 10−2, and 10−3 were accomplished and the amount of S. mutans recovered was determined by plate count using Colombia blood agar.

Statistical analysis: Statistical analysis was carried out using one-way analysis of variance (ANOVA). LSD test was used to calculate the significant differences between tested mean, the letters (A, B and C) LSD represented the levels

of significant, highly significant started from the letter (A) and decreasing with the last one.

3. Result

For the agar well technique, the diameter of the inhibition zone in mm for each group is summarized in Table 1, which shows that group (III)where both Er,Cr:YSGG and CHX were used exhibited greatest diameter of inhibition zone). Group (I), 2% CHX alone, came in second. Er,Cr:YSGG laser group (II) showed the smallest inhibition zone diameter.

Table (1) Diameter of inhibition zones in mm measured in each group

Tested groups	N	Minimum	Maximum	Mean /mm	Std. Error	Std.D
Group I CHX	7	15.	22.	B 18.71	.94	2.49
Group II Laser	7	8.	12.	C 10.26	.56	1.49
Group III CHX+ laser	7	21.	32.	A 26	1.5	4.
P value	P0.001					

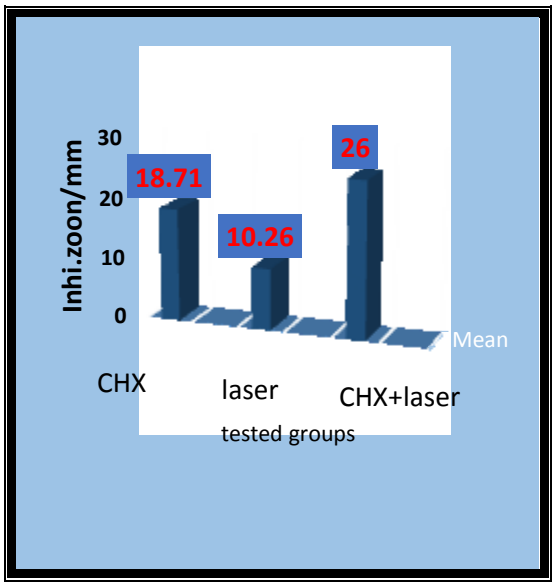


Figure (2) bar chart displays the inhibitory zone diameter for strep. mutans in millimeters

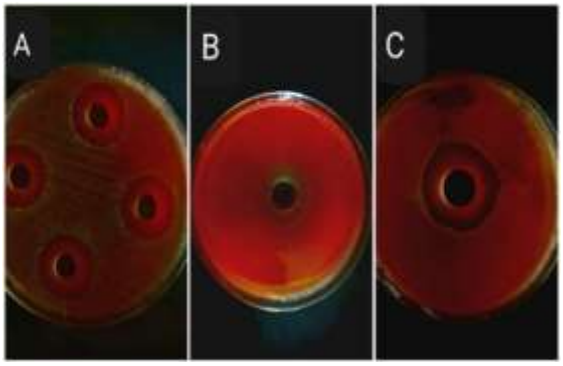
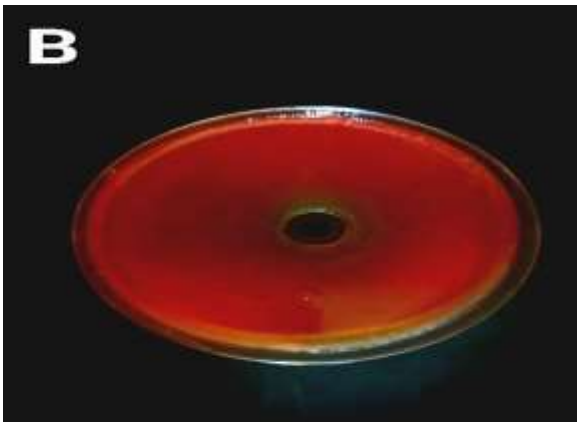


Figure (3) A) CHX, **B)** Laser and **C)** CHX+Laser



Figure(4) Inhibition Zone of Laser

Table 2 provides a summary of descriptive analysis regarding the number of thecovered bacteria across the groups. It demonstrates that the control group (A) had the highest mean percentage, which corresponds to the highest number of recovered bacteria. This was followed by group (B) which used 2% CHX as a cavity disinfectant, and finally by group (C) which utilised erbium laser alone. The activated erbium laser group with a 2% CHX group showed the lowest mean percentage, which was detected (D)

A) Conventional treatment, B) 2% chlorohexidine gloconate, C) Er,Cr:YSGG induced photoaccusic steaming, D) Er,Cr:YSG photoaccustic streaming with 2%CHX .

Table 2: Descriptive regarding the number of the covered bacteria across the groups.

Descriptive Statistics						
Tested groups	N	Minimum	Maximum	Mean		Std. Deviation
	Statistic	Statistic	Statistic	Statistic	Std. Error	Statistic
control before/***10^6**	7	.50	6.00	4.79	0.77	2.04
control after/***10^6**	7	10.00	22.00	13.14	1.74	4.60
CTX before/***10^6**	7	5.00	22.00	9.86	2.26	5.98
CTX after/***10^4**	7	.60	10.00	3.44	1.69	4.48
Laser before/***10^6**	7	10.00	20.00	13.86	1.71	4.53
Laser after/***10^3**	7	1.30	7.00	3.54	0.76	2.00
CHX+laser /before***10^6**	7	11.00	45.00	20.86	4.49	11.87
CHX+laser /after***10^2**	7	11.00	19.00	16.00	1.05	2.77

4. Discussion

In a recent study, the bactericidal impact of the Er,Cr:YSGG photon-induced photoacoustic streaming (PIPS) approach at short pulse duration (60 s) was investigated on the strain of Strep. Mutans. According to the results of the statistical analysis, the diameter of the inhibition zone was found to be highest in group D, which utilized both CHX and laser (table 1)

The primary reason may be that the chemical disinfectant absorbs laser energy, which results in the production of reactive oxygen species that disrupt the bacterial membrane (Yao *et al*, 2012). This, in turn, leads to an increase in the invasion of CHX particles into the bacteria, which ultimately results in the bacteria's death.

Because the laser beam is collimated and focusable, which means that all of the light rays or waves are travelling parallel to each other with the smallest divergence, the diameter of the inhibition zone in group C, where the Er,Cr:YSGG laser-induced photoacoustic streaming was used to inhibit the streptococcus mutants bacteria, is the smallest. This is because the laser beam is collimated and focusable (Menzel, and Photonics, 2013).

The descriptive statistics showed that the mean values of the percentage in the three experimental groups were lower than the control group. The percentage of CFU in group D where Er, Cr: YSGG photon induced photoacoustic streaming with 2% CHX was significantly high, followed by group C where Er,Cr:YSGG laser-induced photoacoustic streaming was used alone, while in group B that used CHX alone, less inhibition was observed than both Erbium laser groups (table 2).

In a previous study, the Er, Cr: YSGG laser was used at 0.75 and 1 W of output power and 20 Hz repetition rate, which produced statistically similar disinfectant potential in cavity walls to the use of chlorhexidine gluconate-based disinfectant solution (Turkun *et al*, 2006). However, in the current research, a better result was gotten in group C using the PIPS technique (table2), it could thus be assumed that the activation of CHX using PIPS by Er,Cr:YSGG laser provide the best antimicrobial activity compared to the conventional method .the main cause could be the photomechanical effect that occurs when the laser light energy is pulsed in a liquid (Sabreen and Hussien *et al* 2021; De Groot *et al*, 2009), When activation takes place in a liquid with minimal volume, the Er,Cr: YSGG frequency absorbed in water, with its peak power that after short pulse (60 sec), may be the reason for the observed photomechanical phenomenon. The resulted phenomenon was the reason for lowering the bacterial content of the treated cavities in group D, where the CHX agitated by erbium laser.

Based on the descriptive analysis, the group B with CHX only showed the lowest percentage of inhibition; this is because Berutti et al. (Berutti *et al*, 1997) found that chemical antimicrobials only reached 130 m into the dentin. In lab experiments, the length of time samples are inoculated has a direct impact on the depth of bacterial penetration. The cavities in the current investigation were only infected for 72 hours. This time frame is typically significantly longer in clinical situations, and the depth of bacterial penetration may even be greater than in our samples. In this regard, the deeper laser beam penetration depth provides a benefit in the removal of bacteria discovered in deeper layers of dentin during dental treatment.

The Er, Cr: YSGG laser was used in the current investigation with 600 um diameter glass tips (MZ6) and sub-ablative conditions (power of 0.25W, 15Hz, with 1% water and 1% air) at short pulse (60 sec). Before being exposing the teeth to laser radiation , rotary instruments were used for cavity preparation, that is because the laser parameter used in this study were significantly different from those typically used in clinical application due to the specific need of this microbiological investigation. The power output employed in this investigation to disinfect the cavities were lower than those used

for cavity preparation. Since the concentration of bacteria is highly sensitive to its diameter, the cavities prepared manually with round bur to guarantee that all of the cavities were of a uniform size. Additionally, water cooling aided by a water spray must be utilized cautiously to avoid the risk of transferring the microbes to other surfaces, since doing this will diminish the bacterial concentration inside the cavity and result in false negative results.

The antibacterial effect appears to be better than the conventional approach. Taking into account these factors, the bacterial inhibition was accomplished through photomechanical flowing of liquid as a result of laser activation rather than usual thermal vaporization. This light energy phenomenon is known as photon-induced photo acoustic streaming (PIPS), which produces shock waves. These shock waves are violent and very quick, which causes the bacterium cell wall to abruptly disintegrate.

In the current study the antibacterial effects of Er,Cr:YSGG laser and Concepsis(CHX) assessed by the cavity tooth model test designated by Özer et al. (Özer *et al ,2003*) .For the comparison of antibacterial activity of different materials, other antibacterial activity test models like the agar well and disc diffusion techniques considered to be inappropriate , as the diffusion rate of antibacterial solutions into the hydrophilic agar may differ meaningfully thus effecting the result. The cavity tooth model was developed to overawed these difficulties and to be able to compare materials by more accurate scientific reproductions, (Özer *et al, 2003*).

5. Conclusions

conclusion: From the extracted result, a photon-induced photoacoustic streaming technique employing an Er,Cr:YSGG pulsed laser (0.25 watts, 15 hertz, 1% air, and 1% water) at short pulse duration (60 microsecond) effectively agitates a chlorhexidine-based cavity disinfectant, which leads to the inhibition of Streptococcus Mutans.

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تطهير التجويف السني بأستخدام تقنية التدفق الضوئي – الصوتي المحتث بليزر الاربيوم – كروميوم

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الخلاصة

مقدمة: الهدف دراسة تأثير تقانة الري بالتدفق الصوتي - الضوئي المحتث بليزر الاربيوم – كروميوم في مدة النبضة القصيرة (٦٠ مايكروثانية) على عدد بكتريا المكورات العقدية الطافرة في المختبر.
المواد والطرق: تم استخدام ثمانية وعشرين ضرس ثالث مقلوع خالية من التسوس والشقوق والعيوب الأخرى. تم اختبار المواد باستخدام تقنية آجار جيدا ونموذج تجويف الأسنان. تمت تعبئة مواد الاختبار في آبار الألواح الملقحة بالعقدية الطافرة. بعد ٤٨ ساعة من الحضانة ، تم قياس مناطق المثبطات بالمليمترات. بالنسبة لاختبار نموذج تجويف الأسنان ، تم تحضير تجاويف أسطوانية في السطح الإطباقي المسطح للأسنان. تركت الأسنان في مزرعة مرق بكتريا المكورات العقدية الطافرة عند درجة ٣٧ درجة مئوية لمدة ٧٢ ساعة. ثم تم تقسيم الأسنان بشكل عشوائي إلى أربع مجموعات من سبعة أسنان (١٤ مجموعة تجويف) لكل منها. في المجموعة (أ) ، تُركت التجاويف التجريبية دون معالجة للسيطرة. في المجموعة (ب) ٢٪ مطهر تجويف قائم على الكلورهيكسيدين مطبق في تجاويف الزفير لمدة ٦٠ ثانية. في المجموعة (ج) تم استخدام ليزر الاربيوم – كروميوم (٠،٢٥واط، ١٥ هيرتز ، ١٪ هواء ، ١٪ ماء) بمدة نبضة قصيرة (٦٠ مايكرو ثانية). في المجموعة الأخيرة ، تم استخدام مطهر تجويف الكلورهيكسيدين لمدة٦٠ ثانية ثم الليزر لمدة ٣٠ ثانية بنفس المعلمة المذكورة أعلاه. ظلت الأسنان في محلول ملحي لمدة 72 ساعة. تم الحصول على كميات قياسية من رقائق العاج من جدران التجويف وتم حساب عدد البكتيريا المستعادة. النتيجة: تم تحليل النتيجة باستخدام اختبار ANOVAو .LSD بعد الاختبار الإحصائي، لوحظ فرق كبير في قطر منطقة التثبيط في المجموعة د (٢٦ مم) حيث تم استخدام كل من الكلورهيكسيدين والليزر متبوعًا بالمجموعة ب(١٨،٧١مم) حيث تم استخدام مطهر التجويف القائم على الكلورهيكسيدين. أقل فرق معنوي لوحظ في المجموعة ج (١٠،٢٦مم) حيث تم استخدام الليزر وحده. الخلاصة: بناءً على هذه الدراسة في المختبر ، فإن تقنية التدفق الضوئي الصوتي المحتث بالفوتون باستخدام الليزر الاربيوم -كروميوم (٠،٢٥ واط، ١٥ هيرتز، ١٪هواي، ١٪ ماء) عند مدة النبضة القصيرة (٦٠ مايكرو ثانية) يعمل على تحفيز مطهر التجويف القائم على الكلورهيكسيدين بشكل فعال مما يؤدي إلى تثبيط المكورات العقدية الطافرة.