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Efficiency of Pulsed Er, Cr: YSGG Laser Induced Photoacoustic Streaming as a Cavity Disinfectant against *Streptococcus mutans*

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ABSTRACT

Aim: the goal of this study was to determine the antibacterial effectiveness of photon induced photo acoustic streaming using Er, Cr:YSGG laser at short pulse duration against heavily colonized teeth with streptococcus mutans in vitro.

Material and Methods: in this study we used twenty eight extracted third molars free of caries, cracks, restoration and other defects. In order to conduct the tooth cavity model test, cylindrical cavities were prepared in the flat occlusal dentin of the teeth. The teeth were stored at 37°C for 72 hours in a broth culture of Streptococcus mutans allowing bacterial invasion. The teeth were then distributed into four groups of seven teeth (14 cavity preparations). The cavities in the first group were left untreated as a control. In the second group the chlorhexidine gluconate–based cavity disinfectant were applied according to the manufacturer's instructions. The erbium, chromium doped yttrium –scandium –gallium garnet laser (0.25 W,15 Hz, 1% air, 15 water) at short pulse duration (60 µ sec) applied to the experimental cavities of the third group. Lastly the experimental cavities in the fourth group both chlorhexidine and laser were applied (chlorhexidine used first then the laser) with the same parameter used above. The teeth were kept in saline for 72 hours. The colony forming unit was counted after collecting standardized amount of dentin chips.

Result: The results were analyzed by ANOVA, Dunnett t and LSD tests. There was statistically a significant difference in the streptococcus mutans count before and after the treatment (P<0.05). In terms of antibacterial efficiency, photon induced photo acoustic streaming by Er,Cr:YSGG laser with 2% Chlorhexidine was found to be the most efficient disinfection method (47841.14286), followed by, photon induced photo acoustic streaming using Er,Cr:YSGG laser alone(47821.71429), 2% Chlorhexidine -gluconate based cavity disinfectant alone(45391.42857) respectively.

conclusion: the result of this in vitro study showed that, photon induced photo acoustic streaming by pulsed erbium, Chromium doped yttrium scandium gallium garnet laser (0.25 W,15Hz,1% air, and 1% water) at short pulse duration (60 µ sec) effective in lowering the number of the recovered bacteria, especially when used to together with chlorhexidine - gluconate based cavity.

Key words: Er, Cr: YSGG laser, PIPS, Streptococcus mutans, Disinfection

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INTRODUCTION

The main objective of caries removal is to overcome the persistent inflammation and treatment failure. That caused by the remnant of infected and necrotic tissues and microorganisms thus, complete removal of the infected dentin related directly to the clinical success of a restoration. However, the caries treatment procedures used nowadays not assuredly remove all the form of the microorganisms that present in the tooth surface [1,2]. By solely mechanical means, attempts at the complete removal of deep carious dentin, may result in pulpal damage or gross destruction of the tooth structure [3,4]. In an effort to reduce or eliminate bacteria during cavity preparation, multiple disinfectants have been used in clinical dentistry. Some of these agents have been reported to cause pulpal violation, due to their innate chemicals, therefore have been neglected [5].

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 [6]. Since the chemical disinfectant may alter the ability of hydrophilic resins to seal dentin tubules, there are some worries

about the use of them before the application of dentin bonding agents [7-9]. These worries are compounded by the many contentious results arising from various studies on the interaction between cavity disinfectants and dentin bonding agents [7,9,11].

Much less consideration has been given to pathogens associated with dental caries when compared to the widespread investigation into the antibacterial effects of different lasers including Er,Cr:YSGG laser, on endodontic and periodontal pathogens, Türkün, et al. [12] have inspected the antibacterial activity of Er, Cr:YSGG laser against *Streptococcus mutans*. The researchers compared the antibacterial effects of Er, Cr:YSGG laser with 0.75 and 1 W power outputs and chlorhexidine gluconate-based cavity disinfectant against *Streptococcus mutans* and found that the antibacterial activity on *Streptococcus mutans* established by Er, Cr:YSGG laser with both energy outputs was parallel to that of the chlorhexidine.

Laser energy may not only kill bacteria directly (theoretically), but also activate the irrigant to enhance its bactericidal actions [13,14]. 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 [15]. This light energy phenomenon described as photon induced photo acoustic streaming (PIPS) [16]. The photomechanical flowing of the liquids will efficiently remove the smear layer by a non-thermal manner, which can avoid the undesired effects of thermal energy [17].

The current study aimed to investigate the bactericidal effect of Er,Cr :YSGG laser at short pulse duration (60 µ sec) with and without CHX chemical disinfectant next to shock wave generation for photon induced photoacoustic streaming (PIPS) technique.

MATERIAL AND METHODS

The Department of Basic science and Microbiology of University of Baghdad provide *Streptococcus mutans* to estimate antibacterial activity of test materials. The techniques used in the recent experiment were the tooth cavity model.

Tooth cavity model technique - According to the method used by Özer, et al. this part of the study was accomplished [18]. 28 human molars free from caries, restorations, or other defects were used for that purpose. They were stored in distilled water at 4°C immediately after extraction. 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 producing pulp exposure as show in Figure 1.

Teeth were randomly dispersed into the four groups of seven teeth (14 cavity preparations) each. To confirm sterility, the teeth were sterilized by an autoclave for 15 minutes at 121°C. the teeth were put into brain heart infusion (BHI) broth and incubated for 24 hours at 37°C. Each tooth was transferred in an individual tube hold 2 ml of sterile physiologic saline (SPS), for washing out the culture medium and to avoid dehydration 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, altogether teeth were placed in a bottle containing broth culture of *Streptococcus mutans* suspension (106 CFU/ml) and incubated at 37°C for 72 hours.

The teeth were taken out from the bottle, and the cavities were desiccated again with sterile paper points and a gentle stream of air, Following incubation.. In Group A, the cavities were left untreated and functioned as the control. In Group B, the CHX 2% was applied into the cavities using a sterile brush, left untouched for 60 seconds, and then dried with an air-syringe. In Group C, Laser light was used as the bactericidal agent. Er,Cr:YSGG laser Waterlase, Biolase, California, USA

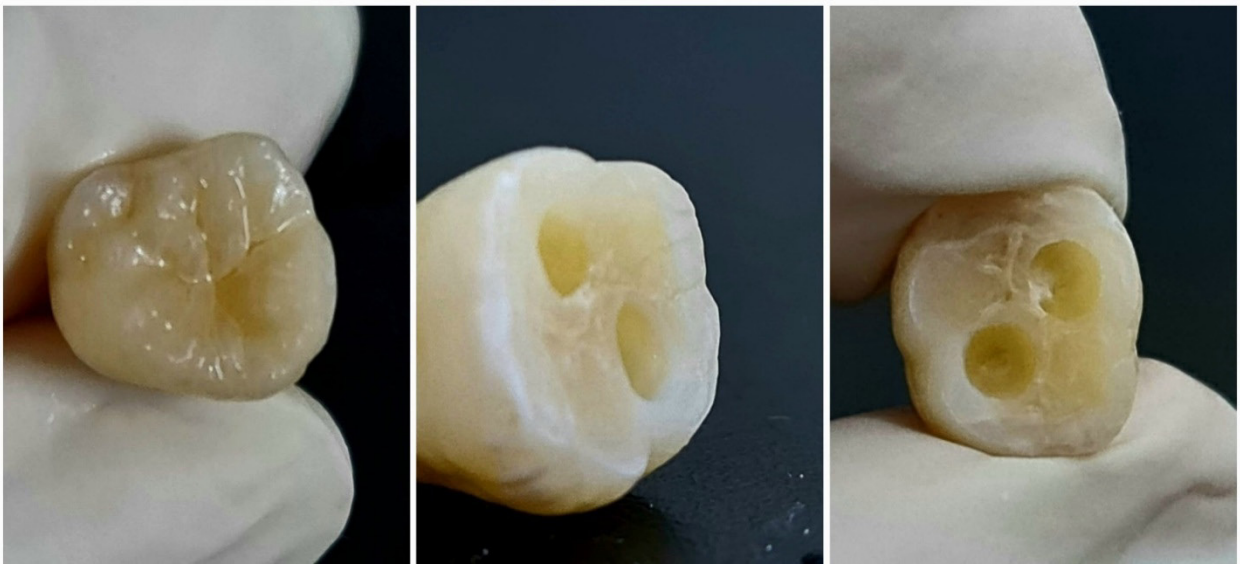

Figure 1: Preparation of control and experimental cavities.

at short pulse duration (60 µ sec) with 0.25 W power outputs, 1% water jet and 1% air jet was applied to the experimental cavity for 30 s. 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.

The teeth were saved in SPS separately after sticking the occlusal surfaces with a temporary restorative material (Cavit GC) at 37°C for 72 hours. The teeth were then removed from the SPS. Using a new sterile steel bur, mounted to low-speed contra-angle hand piece identical amounts of dentin chips (20 ± 5mg) were collected from the cavity, and then put 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 *Streptococcus mutans* recovered was determined by plate count using Colombia blood agar.

Statistical analysis: The statistical analysis was carried out using one-way ANOVA to compare various groups with each other. Results were expressed as mean+standard error (SE) and values of p>0.05 were considered statically non-significant. 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. Dunnett t-tests treat one group as a control, and compare all other groups against it. Similar letters mean there are no significant differences between tested mean. The statistical analysis was carried out by SPSS (v 20).

RESULTS

Table 1 display the summary of descriptive statistics of the number of the recovered bacteria amongst the groups, and demonstrate the highest mean percentage (the highest number of recovered bacteria)was presented in the control group (A) followed by group(B) that use 2% CHX as a cavity disinfectant then group (C) erbium laser alone. The lowest mean percentage observed in activated erbium laser group with 2% CHX group (D) A) conventional treatment, B) 2% chlorhexidine gloconate, C) Er,Cr:YSGG laser PIPS, D) Er,Cr:YSGG laser PIPS with 2% CHX.

Table 2 shows that the high mean difference was between the control group and the activated Erbium laser group with 2% CHX, followed by Erbium laser group (C) and the 2% CHX group (B) Dunnett Test was used to compare the mean of control group against the mean of the experimental groups un order to study the difference.

Figure 2 bacterial count values of *Streptococcus mutans* in percentage amongst all four groups and show that the percentage of *Streptococcus mutans* inhibition increased in activated erbium laser group (C) then become highest in activated erbium group with 2%CHX (D) and there were some reduction in percentage when 2%CHX used alone (B).

DISCUSSION

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. [18]. 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 [18,19].

Table 1: Descriptive statistics.

Tested groups	N	Minimum	Maximum	Mean		Std. Deviation
	Statistic	Statistic	Statistic	Statistic	Std. Error	Statistic
A(control)before/*10^6	7	0.5	6	4.79	0.77	2.04
A(control)after/*10^6	7	10	22	13.14	1.74	4.6
B before/*10^6	7	5	22	9.86	2.26	5.98
B after/*10^4	7	0.6	10	3.44	1.69	4.48
C before/*106	7	10	20	13.86	1.71	4.53
C after/*10^3	7	1.3	7	3.54	0.76	2
D /before*10^6	7	11	45	20.86	4.49	11.87
D /after*10^2	7	11	19	16	1.05	2.77

Table 2: Dependent variable: CFU. Dunnett t (2-sided).

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
CHX	Control	45391.42857	5530.5687	0	-59254.7435-	-31528.1137-
LASER	Control	47821.71429	5530.5687	0	-61685.0292-	-33958.3994-
CHX+LASER	Control	47841.14286	5530.5687	0	-61704.4577-	-33977.8280-

*The mean difference is significant at the 0.05 level

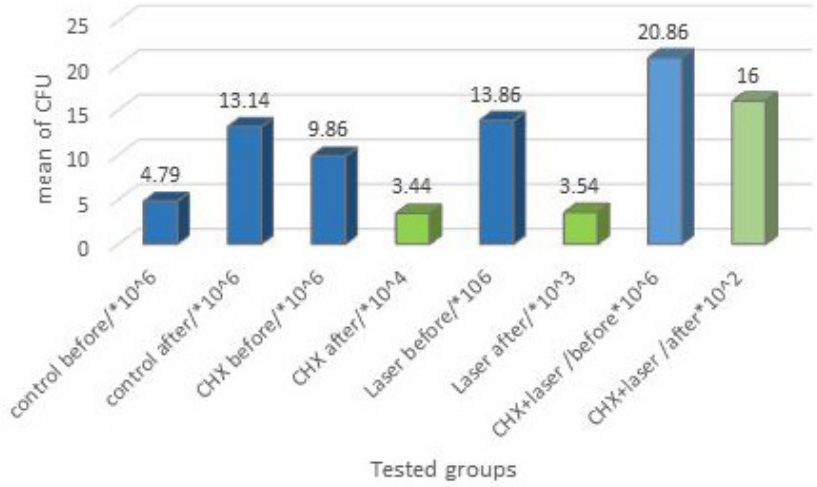

Figure 2: Bar chart showing the mean percentage of CFU of the study groups.

The existing study explores the bactericidal effect of Er,Cr:YSGG photon-induced photo acoustic streaming (PIPS) technique at short pulse duration (60 µs) with powers (0.25 W) on *Streptococcus mutans*. Taking attention that the power of 0.25 W represents the beginning of shock wave generation (chemical disinfectant activation). That gained from the pilot study. The statistical analysis exposed that the mean values of percentage of CFU in group D (laser with CHX) were significantly high (Table 1), as well as the mean difference between the control group (A) and the activated laser group (D) was significantly high (47841.14286),it could thus be assumed that the activation of CHX using PIPS by Er,Cr:YSGG laser deliver the greatest antimicrobial activity related to the conventional method. The chief reason could be the photomechanical effect that happens when the laser light energy is pulsated in a liquid [20,21], the Er,Cr:YSGG wavelength absorbed in water, with its peak power after the short pulse (60 µsec) may cause the photomechanical phenomenon that detected once the stimulation occurs in a liquid with lowest bulk. The occasioned phenomenon was the cause for dropping the bacterial content of the treated cavities in group D,where the CHX stimulated by erbium laser.

In a prior study that use the Er,Cr:YSGG laser at 0.75 and 1Woutput power and 20 Hz repetition rate lead to a statistically parallel disinfectant potential to the use of chlorhexidine gluconate-based disinfectant solution [14]; however, in the present study, a better results were achieved by using PIPS technique.

The mean difference of CFU between the control group (A) and group (C) was (47821.71429) (Table 2) in group (C) where the laser radiation alone used with PIPS for shock wave generation and decontamination

In the second group that use CHX alone the lest percentage of inhibition attained compared to both Erbium laser groups (Table 1). Since the chemical disinfectants penetrated no more than 130µm into the dentin as showed by Berutti, et al. [22]. On the other hand In laboratory explores, the inoculation period of samples also in a straight line affects bacterial penetration depth.. In the extant study, the cavities were inoculated for only 72 hours In clinical situations, the incubation period is generally much longer and bacterial penetration depth may be deeper than that of our models. Subsequently, the deeper penetration depth of laser beam is a favorable advantage in the elimination of microorganisms found in deeper layers of dentin during dental treatment..

It was also distinguished that these outcomes influenced by the outline of the cavity in addition to different belongings of the material and the time of application. In our study, we used cylindrical cavities of 1-mm diameter and 2-mm depth as an alternative to flat surfaces to mimic clinical situations. The antibacterial effect of the concepsis is applicable only on the decontaminated surfaces Thus, we believe that during the concepsis application any contaminated surface not touched by chemical disinfectant would cause an increase in the amount of recovered bacteria owing to the imitation of microorganisms, at this point the advantage of the shock waves that will impulse the chemical disinfectant to the restricted extents that cannot be grasped conventionally.

The Er,Cr:YSGG laser PIPS in the current study was conceded with 600 um diameter glass tips(MZ6) by with sub ablative parameters(power o.25W,with 1%water and1% air) at short pulse (60 µsec), the gotten antimicrobial effect seem to be improved compared to the conventional method. Considering these parameters, the bacterial inhibition was by funds of photomechanical flowing of liquid due to laser stimulation not by typical thermal desiccation, this light energy manifestation is referred to as photon-induced photo acoustic streaming (PIPS) which produce a shock waves, these shock waves are ferocious and very fast lead to sudden collapse of the bacterial cell wall.

Parameters of the laser energy used in this study were rather different from the parameters generally chosen in clinical applications. This was so basically due to the necessities of this microbiological study. For instance, the cavities for bacterial inoculation were equipped by

rotary instruments before irradiation. Hereafter, the power outputs used in this study to disinfect the cavities were lesser than that used for cavity preparation in clinical applications. If high laser energy is used to irradiate the tooth surface, it will cause carbonization and flaws on the laser beam applied exterior [23].

CONCLUSION

From the extracted results, PIPS technique using pulsed Er,Cr:YSGG laser(0.25 W,15Hz,1% air, and 1% water) at short pulse duration (60 μsec) evidenced to be an effective in augmenting the efficiency of chlorhexidine based cavity disinfectant in reduction the number of the recovered bacteria and inhibiting new bacterial growth.

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