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Antibacterial effect on *Enterococcus faecalis* of erbium, chromium: yttrium-scandium-gallium-garnet laser irradiation compared to two irrigating solutions in root canals of extracted human teeth

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Abstract

Objective To compare the antibacterial effect of the erbium, chromium: yttrium-scandium-gallium-garnet (Er,Cr:YSGG) laser irradiation with two irrigating solutions in root canals of extracted human teeth.

Materials and methods One hundred and twenty-five extracted single-rooted teeth were collected. The canals were then enlarged with K files to size 50 using crown-down technique and randomly assigned into four experimental groups of 30 teeth each and five teeth for sterility control group. After sterilization, all roots except the sterility control group were inoculated with 10 microlitres of a known concentration of *Enterococcus faecalis* ATCC29212 and incubated at 37° C for 48 hours. The first group was used as a negative control receiving no treatment. The second group and third group were irrigated with 2.5% sodium hypochlorite (NaOCl) solution and 2% chlorhexidine (CHX) solution for 10 minutes

respectively. The last group was irradiated with the Er,Cr:YSGG laser at 1.5 watts output power with no air and water using four lasing cycles of 10 seconds each. After treatment, sterile normal saline solution was filled into the canals and the walls were then circumferentially filed with H-file size 50. The content was then transferred and plated on tryptic soy agar immediately. All plates were incubated at 37°C for 24 hours. The colony-forming units were counted, and the quantitative results were subjected to an One-Way ANOVA test and Tamhane's Test.

Results The differences in the mean number of viable colonies between the control and the other groups were statistically significant different ($p < 0.05$). Comparing among the treated groups, the mean Log colony forming units values obtained after Er,Cr:YSGG laser irradiation were statistically significantly higher than 2.5% NaOCl and 2% CHX group ($p < 0.05$). However, there was no significant difference between the 2.5% NaOCl and 2% CHX group ($p > 0.05$).

Conclusion It can be concluded that Er,Cr:YSGG laser irradiation can reduce the viable microbial population in root canals to a certain extent but is less effective than irrigating with 2.5% NaOCl and 2% CHX solutions.

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Key words: *chlorhexidine; Enterococcus faecalis; erbium, chromium: yttrium-scandium-gallium-garnet; laser irradiation; sodium hypochlorite*

Introduction

Bacterial infection plays an important role in the development of necrosis in the dental pulp and the formation of periapical lesions.[1] The persistence of bacteria in the root canal system after endodontic treatment may cause persistant inflammation in the periradicular tissue and often leads to failure.[2] Accepted treatment procedures to eliminate the infection include a combination of chemical cleaning involving irrigation with a disinfecting agent and mechanical instrumentation. The most popular irrigating solution is sodium hypochlorite (NaOCl). It is an effective antimicrobial agent[3] and an excellent organic solvent for vital, necrotic and fixed tissues. Sodium hypochlorite dissolves proteins and forming chloramines residues on the remaining peptide fragments, thus not only aiding in debridement but also contributing to antimicrobial action of the free chlorine. Furthermore, it inactivates the sulfhydryl groups of bacterial enzymes by forming hypochlorous acid.[4] However, it is highly irritating to periapical tissues especially at high concentrations.[5,6] Chlorhexidine (CHX) has been recommended as a root canal irrigant and medicament. It is a potent antimicrobial agent and has a low grade of toxicity. CHX seems to act by adsorbing onto the cell wall of microorganisms and causing leakage of intracellular components.[7] However, CHX is unable to dissolve pulp tissue and may remain on canal walls, obstructing the dentinal tubules.[8]

Enterococcus faecalis is known to be one of the predominant bacteria in teeth which root canal therapy fails and appears to be highly resistant to medicament used during treatment.[9,10] Despite the use of antimicrobial chemicals for irrigation, the existence of accessory canals, anastomoses and fins creates a three-dimensional network that makes the complete elimination of debris and achievement of a sterile root

canal system difficult.[11] In order to achieve better results of endodontic treatment, a great deal of effort has been made to find another approach. New approaches to eliminate the infection from root canal systems include laser technology.[12,13] The antibacterial effect of a laser beam is based on thermal properties of the laser tissue interaction.[14] Dental lasers could provide greater accessibility to formerly unreachable parts of the tubular network because of their enhanced penetration into dentinal tissues[15,16] and consequently may have antimicrobial effects to aid in the reduction of bacteria in the root canal.[17-19]

The Er,Cr:YSGG laser at a wavelength of 2.78 μm has become available in the field of laser-assisted endodontics. It is a laser system unit approved by the U.S. Food and Drug Administration for cleansing, shaping and enlarging the root canal.[20,21] The Er,Cr:YSGG laser system uses hydrokinetic energy-the laser energy heats the air and water directly in front of the atomized water molecules with the aim of accelerating them to a higher speed. Thus, the Er,Cr:YSGG laser may have a greater ability to disinfect root canals.

Former studies on the Er,Cr:YSGG lasers seemed to focus on caries removal and cavity preparation, and little is known about its bactericidal effectiveness.[22] Moreover, there are only a few studies that compared the antibacterial efficacy of the Er,Cr:YSGG laser irradiation in infected root canal with irrigating solutions.[23]

The objective of this study was to compare the antibacterial effect of Er,Cr:YSGG laser irradiation with 2.5% NaOCl and 2% CHX irrigation when used in the root canals that were infected with *E. faecalis.*

Materials and methods

Laser device

The Er,Cr:YSGG laser (Waterlase Millenium; Biolase Techn., San Clementa, CA, USA) was used in this study. This laser operates at a wavelength of 2.78 μm with a pulse energy that can be varied between 25 and 300 mJ at a fixed repetition rate of 20 Hz. This results in an output power of 0.5-6 W. An automode was used in this study with 1.5 W output power with no water and air spray.[24] The laser beam was delivered via a 200 μm endodontic fiberoptic tip.

Sample preparation

One hundred and twenty-five extracted human single canal-rooted teeth were stored in saline solution until employed in the experiments. The coronal portion was removed to the cemento-enamel junction using a diamond fissure bur to obtain root canal length of 15 mm. The pulp was removed and the working length of each root canal was established at 1 mm short of the apical foramen with a K file size 20 (K-type file; Mani Inc., Nakaakutsu, Japan). Instrumentation was completed with K-file to size 50 using crown-down technique. Sterile physiological saline was used as an irrigating solution after the completion of each file size. The apical foramen was then closed with flowable composite resin (Z 350, 3M ESPE, Thailand), and the root surface was sealed with two coats of nail polish. The smear layer was removed by the sequential irrigation of 5 ml of 17% ethylene diamine tetraacetic acid (EDTA) and 5.25% NaOCl for 3 min each. All teeth were individually placed in plaster blocks for ease in handling and the orifices were closed with aluminium foil. The sterility of all root canals were achieved by autoclaving at 121°C for 15 min. The sterility was confirmed by culturing of samples using sterile paper points and incubated in Tryptic soy broth at 37°C for 24 h. Any sample showing turbidity was discarded.

Bacterial inoculation

E. faecalis ATCC 29212 was used in this study. Before starting the experiments, the frozen (-20°C) bacterial sample was thawed and grown for 24 h on a solid culture medium (Mitis salivarious agar) at 37°C

under aerobic conditions. Five bacterial colonies were then placed in 25.0 ml of tryptic soy broth and incubated for an additional h at 37°C for 24 h under aerobic conditions. The purity of the strain was confirmed by Gram's stain. The cell suspension was adjusted to 10^8 colony forming units per ml (CFU/ml) as determined by OD 550 nm.

All teeth were randomly divided into four groups of 30 teeth and one group of five teeth to be used as the sterility control group.

Ten µl of the bacterial culture were transferred into the lumen of the mechanically enlarged root canals using a sterile micropipette (Eppendorf, Hamburg, Germany) and the orifices were closed with sterile aluminium foil. Then, all samples including the sterility control group were then incubated at 37°C for 48 h.

Canal treatment

After 48 h, all canals were dried with sterile paper points. The canal in the sterility control group was filled with 10 µl of fresh medium as a control of the contamination and leakage of the sample during the experiment. The canals in the second and the third group were irrigated with 5.0 ml of 2.5% NaOCl and 2% CHX solution, respectively. The solutions were delivered to within 1 mm from the working length using sterile 5.0 ml plastic syringes and 25-gauge needles. After allowing the solutions to fill the canals for 10 min,[25] the canals then were irrigated with 2 ml of distilled water. In the laser group, each root was irradiated with the Er,Cr:YSGG laser (Waterlase Millennium; Biolase Tech., San Clemente, CA, USA), using 1.5 W output power. The laser beam was delivered via an endodontic fiberoptic tip (Milennium; Biolase Technology Inc. (P/N 5000602)), diameter of 200 µm. The fiber tip was inserted into the root canal to within 1 mm from the working length. The laser was activated and the tip was slowly moved in a helicoidal manner from the apical to the cervical part of the canal for 10 s period with 15 s of rest between each lasing cycle. The total irradiation time was 40 s per canal.[24]

Bacterial analysis

After treatment, the liquid contents of root canals of all groups were carefully absorbed with sterile paper points without intentionally touching of the walls. All of the root canals were then filled with 10 µl sterile normal saline and gently circumferentially filed with sterile H-file size 50 at 1 mm short of the apex for 20 s. Then, the contents inside the canal were transferred using a sterile micropipette and immediately plated on Tryptic soy agar and incubated for 24 h under standard aerobic conditions. The content from the negative control group was diluted a hundred folds before being plated on the agar. CFU were counted. The purity of the strain was confirmed again by Gram's stain.

Statistical analysis was performed using the SPSS program for Windows 15.0 (SPSS Inc., Chicago, IL, USA). The logarithm of CFU (Log CFU) values were subjected to One-way ANOVA test for significant difference ($\alpha = 0.05$). The Tamhane's Test was used for group comparison ($\alpha = 0.05$).

Results

The number of samples with no growth of bacteria observed are shown in Table 1. The negative control group had the bacterial growth in all samples (30 out of 30 samples). Among the treated groups, the CHX irrigation gave the highest number of sterilized teeth (26 out of 30 samples), and followed by 23 out of 30 samples in the NaOCl irrigation group and none (0 out of 30 samples) in the laser irradiation group.

The Log CFU count was calculated in order to compare the number of remaining bacteria in each group after the treatment. The negative control group had the highest mean Log CFU of remaining bacteria (10.47

± 1.00). There was a statistically significant difference between the negative control group and all other groups ($p < 0.05$). Mean Log CFU values of the NaOCl group and CHX group were 0.40 ± 0.54 and 0.27 ± 0.55, respectively. However, no statistically significant difference was observed between NaOCl group and CHX group ($p > 0.05$). Mean Log CFU values after Er,Cr:YSGG laser irradiation was 5.34 ± 0.78 which was statistically significantly higher than that of the NaOCl or CHX group ($p < 0.05$) as showed in Figure 1.

Discussion

The methodology used in this study followed that described in Eldeniz *et al.*[23], Ramskold *et al.*[26] and Le Goff *et al.*[27] *E. faecalis* is a facultative Gram-positive anaerobic coccus that is a known endodontic pathogen, being frequently recovered from the root canals of teeth associated with post-treatment diseases.[2] In this study, *E. faecalis* infected teeth were incubated for 48 h in order to evaluate cells in the starvation phase rather than growing cells. Additionally, it has been also

Table 1 The number and percentage of samples with no growth of bacteria in each group

Group	Number of samples with no growth of bacteria/total number of samples	Percentage
Negative Control	0/30	0
NaOCl	23/30	76.7
Chlorhexidine	26/30	86.7
Laser	0/30	0

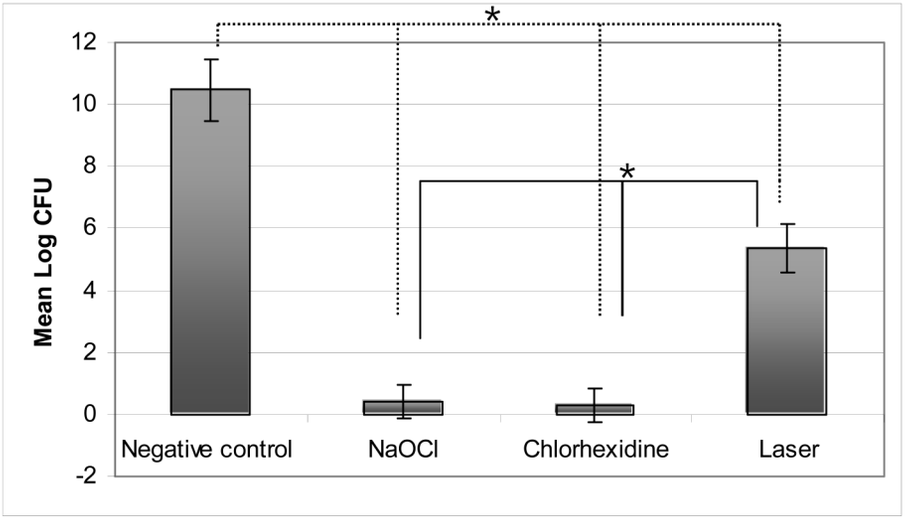

Fig. 1 The comparison of the mean Log CFU in each group. A bar chart represents mean Log CFU of remaining bacteria after treatment in each group. Error bars represent standard deviations.

reported that this microorganism has the ability under specific conditions to infect the whole length of the dentinal tubules within two days.[28]

The time period used for irrigation of the root canals in this study was 10 min which approximately corresponds to the total time required for the biomechanical preparation of a root canal of moderate difficulty. Gomes *et al.*[25] also used 10 min for irrigation in their study to test *in vitro* effect of various concentrations of disinfectant against *E. faecalis.* The microbe was killed in less than 30 s by the 5.25% solution, while it took 10 and 30 min for complete killing of the bacteria by 2.5% and 0.5% solutions, respectively. On the other hand, CHX killed *E. faecalis* in 30 s or less at concentrations of 0.2-2%.[29,30]

The Er,Cr:YSGG laser was used according to the manufacturer's instructions for sterilization and the earlier descriptive procedure was strictly followed.[31] The output power setting of 1.5 W of laser was monitored before starting the experiment. According to the study of Wang *et al.*[24], the Er,Cr:YSGG laser treatment showed a bacterial reduction of 77% after irradiation at 1 W and 96% at 1.5 W with no significant difference. They used 4 cycles of 10 seconds with 15 s of rest between cycles.[24] The previous study of Schoop *et al.*[31] found that the temperature increased 8°C after 1.5 W of Er,Cr:YSGG laser irradiation in the root canals which was in agreement with the study of Yamazaki *et al.*[21]

Our results show that Er,Cr:YSGG laser irradiation significantly reduced *E. faecalis in vitro,* although all samples showed some growth of bacteria. In 2.5% NaOCl and 2% CHX irrigation groups, only a small number of bacteria could be found in the samples after treatment and highly significant reduction of *E. faecalis* when compared to the Er,Cr:YSGG laser irradiation group was demonstrated. The result is in agreement with a previous study which evaluated Er,Cr:YSGG laser.[23] Jha *et al.*[32] concluded that the Er,Cr:YSGG laser instrumentation was not able to completely eliminate *E. faecalis* infection in root canals. Eldeniz *et al.*[23] found that Er,Cr:YSGG laser irradiation did not eradicate all bacteria, whereas 3% NaOCl could inhibit all of *E. faecalis* and was effective to sterilize all root canals. They used 15 min irrigating time for NaOCl which was longer than that used in our study, whereas the 0.5 W output power of laser was lower than that used in this study. In both previous studies, the investigators also recovered residual viable bacteria after the laser treatment of infected root dentin by collecting dentin shavings from the root canal wall as conducted in present study. The antibacterial effect of the laser was found to be less effective than 2.5% NaOCl solution which was in agreement with previous studies.[18,27,33]

The inability of the Er,Cr:YSGG laser to sterilize root canal in this study might be attributed to many reasons. First, with the available fiber optic, the laser beam can only deliver maximum energy to the area perpendicular to the tip where the beam is well focused. Hence, the use of forward fiber tip like this may not be able to perfectly direct the beam against the entire surface of the canal walls at all time. In this study, we tried to repeat a helicoidally movement of the tip from the apical to the cervical part several times during the lasing cycles in order to avoid this limitation. One possibility to improve the bactericidal efficacy of the laser is the development of a new tip that can deliver the laser beam radially. Such a newly designed laser tip, called "radial fiber tip", will be available in the near future to offer predictable, total elimination of viable bacteria in the root canal wall.[34,35] However, further studies are needed to verify its efficiency for its clinical use in infected root canals. Secondly, prior smear layer removal might cause the bacteria to

penetrate deeply in the dentinal tubules or some of them might reside in the ramification of the root canal system and then might be shielded from the laser beam. Other reasons might be the insensitivity of *E. faecalis* to laser irradiation because of its cell wall structure[36] and/or the resistance of starved *E. faecalis* cells to different conditions.[37]

Conclusion

With the limitation of this study, it can be concluded that Er,Cr:YSGG laser can reduce the amount of *E. faecalis* in root canals in a certain extent but is less effective than irrigating with 2.5% NaOCl and 2% CHX solutions. At present, the Er,Cr:YSGG laser could only be considered as a supplement to the conventional protocols for the disinfection of the root canal system. Further improvements are required to increase its antimicrobial efficiency.

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ผลการต้านเชื้อเอ็นเทอโรคอกคัสฟีคาลิสของเออร์เบียมโครเมียมอิตเทรียมสแกนเดียมแกลเลียมการ์เนตเลเซอร์เปรียบเทียบกับน้ำยาล้างคลองรากฟันสองชนิดในคลองรากฟันแท้มนุษย์ที่ถูกถอน

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บทคัดย่อ

วัตถุประสงค์ เพื่อเปรียบเทียบผลการต้านเชื้อของเออร์เบียมโครเมียมอิตเทรียมสแกนเดียมแกลเลียมการ์เนตเลเซอร์กับน้ำยาล้างคลองรากฟันสองชนิดในคลองรากฟันแท้ของมนุษย์ที่ถูกถอน

วัสดุและวิธีการ นำฟันรากเดียวทั้งหมด 125 ซี่ มาเตรียมคลองรากฟันด้วยวิธีคราวน์ดาวน์ให้มีขนาดเท่าตะไบชนิดเค เบอร์ 50 แบ่งฟันโดยการสุ่มออกเป็น 4 กลุ่มๆ ละ 30 ซี่ ที่เหลือ 5 ซี่ใช้เป็นกลุ่มควบคุมภาวะปลอดเชื้อ ภายหลังทำให้ปราศจากเชื้อ ฟันทุกซี่ถูกเพาะเชื้อเอ็นเทอโรคอกคัสฟีคาลิส ที่ทราบความเข้มข้นของเชื้อปริมาณ 10 ไมโครลิตรที่อุณหภูมิ 37 องศาเซลเซียส เป็นเวลา 48 ชั่วโมง ยกเว้นในกลุ่มควบคุมภาวะปลอดเชื้อ กลุ่มแรกเป็นกลุ่มควบคุมผลลบซึ่งไม่ได้รับการล้างคลองราก กลุ่มที่สองและสามได้รับการล้างคลองรากฟันเป็นเวลา 10 นาที ด้วยโซเดียมไฮโปคลอไรต์ความเข้มข้นร้อยละ 2.5 และคลอร์เฮ็กซิดีนความเข้มข้นร้อยละ 2 ตามลำดับ และกลุ่มสุดท้ายได้รับการฉายด้วยเออร์เบียมโครเมียมอิตเทรียมสแกนเดียมแกลเลียมการ์เนตเลเซอร์กำลัง 1.5 วัตต์จำนวน 4 รอบๆ ละ 10 วินาที ใส่น้ำเกลือลงไปในคลองรากฟันและใช้ตะไบชนิดเค เบอร์ 50 ขูดที่ผนังคลองรากฟัน หลังจากนั้นดูดน้ำเกลือในคลองรากฟัน นำไปเพาะบนวุ้นเลี้ยงเชื้อทันทีที่อุณหภูมิ 37 องศาเซลเซียส เป็นเวลา 24 ชั่วโมง นับจำนวนโคโลนีและนำข้อมูลที่ได้มาวิเคราะห์ด้วยสถิติความแปรปรวนแบบทางเดียวและการทดสอบแบบแทมเฮน ที่ระดับนัยสำคัญ 0.05

ผลการศึกษา พบว่าค่าเฉลี่ยของปริมาณแบคทีเรียที่เหลือในกลุ่มควบคุมที่ไม่ได้รับการล้างคลองรากมีจำนวนมากกว่ากลุ่มอื่นอย่างมีนัยสำคัญทางสถิติ ($p < 0.05$) และเมื่อเปรียบในกลุ่มที่ฉายด้วยเลเซอร์และกลุ่มน้ำยาล้างคลองรากฟันทั้งสองชนิดพบว่าค่าเฉลี่ยของปริมาณแบคทีเรียที่เหลืออยู่ในกลุ่มที่ฉายด้วยเออร์เบียมโครเมียมอิตเทรียมสแกนเดียมแกลเลียมการ์เนตเลเซอร์มีจำนวนสูงกว่ากลุ่มที่ล้างด้วยน้ำยาล้างคลองรากฟันทั้งสองชนิดอย่างมีนัยสำคัญทางสถิติ ($p < 0.05$) แต่ในกลุ่มที่ล้างด้วยโซเดียมไฮโปคลอไรต์ความเข้มข้นร้อยละ 2.5 และคลอร์เฮ็กซิดีนความเข้มข้นร้อยละ 2 ไม่มีความแตกต่างกันอย่างมีนัยสำคัญทางสถิติ ($p > 0.05$)

สรุป เออร์เบียมโครเมียมอิตเทรียมสแกนเดียมแกลเลียมการ์เนตเลเซอร์สามารถลดจำนวนแบคทีเรียลงได้ แต่มีประสิทธิภาพด้อยกว่าน้ำยาล้างคลองรากโซเดียมไฮโปคลอไรต์ความเข้มข้นร้อยละ 2.5 และคลอร์เฮ็กซิดีนความเข้มข้นร้อยละ 2

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