Bactericidal Effectiveness of 810 Nm Wavelength Diode Laser: An In-Vitro Study

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Abstract

The aim of the present study was to compare the bactericidal effectiveness of diode laser 810 nm and sodium hypochlorite at 3% on Enterococcus faecalis by using culture and qPCR methods.

34 selected one-root premolars with crowns removed were endodontically prepared with a rotary device, then autoclaved. Two teeth were selected for the negative control group, the remaining 32 teeth were infected with Enterococcus faecalis ACTT 29212 for 21 days. Then two teeth were randomly selected for SEM testing, the rest divided into 3 groups, 10 teeth per group, then the group (1) was filled with saline (control group); the group (2) with 3% sodium hypochlorite at 3% and the group (3) treated with 810 nm diode laser. Next, the sample in the canal was taken with a sterile paper cone and transferred into Eppendorf plastic tube containing 2ml physiological saline. 1ml of diluted product was immediately taken for culture and the remaining 1 ml is used for performing qPCR. The average number of bacteria was collected and transformed into log 10. The t - test was used to compare between groups and between methods. Equalization parity is checked by Levene test.

According to culture results, the average number of bacteria of group (2) was the lowest among the 3 groups, the group (1) was the highest and these differences were statistically significant ($p < 0.001$). According to the qPCR results, the average number of bacteria in the group (2) was the lowest among the 3 groups, the group (1) was the highest and these differences were statistically significant ($p < 0.001$). The average number of bacteria in the 3 groups given by culture method was lower than that of qPCR method and this difference was statistically significant ($p < 0.001$).

Laser diode 810 nm has the ability to kill Enterococcus faecalis, but its effectiveness is lower than 3% sodium hypochlorite solution. qPCR can complement the culture method in endodontic studies in endodontics.

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Introduction

Enterococcus faecalis (*E. faecalis*) is one of the most significant endodontic bacteria because this bacterium has been found in most primary and secondary endodontic lesions [1]. It is identified as the most important factor in cases of endodontic failure [2]. This Gram-positive coccus is capable of persistent survival thanks to its ability to penetrate deep in the dentinal tube. It is also resistant to conventional antibacterial agents, forms a biofilm film, and enters a state that is alive but not viable (non-culturable - VBNC) [3], when conditions are favorable, it causes disease again [4]. An important aspect for the persistence of the bacteria is combined with the complexity of the canal systems [5]. Some areas of the canal become inaccessible during preparation, pumping and filling [6]. Another reason is that the irrigation solutions can only affect depths of about 300 µm while bacteria can survive deeper in the dentinal tube and remain unaffected [7]. Due to the limitations of the conventional chemical-mechanical method in killing bacteria, researchers have been looked for a more effective bactericidal solution. Laser with outstanding advantages has been proven, increasingly used in medicine in general and dentistry because of its ability to kill bacteria

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including resistant bacteria[8]. In endodontics, the first studies using lasers were unsuccessful, but the positive information obtained also contributed to the boosting of further studies[9].

Research on endodontic sterilization today still use the culture method, although it is the gold standard to quantify microorganisms, this method has many disadvantages: time-consuming, highly dependent on technicians and laboratory ... New techniques based on molecular biology such as Polymerase chain reaction (PCR), especially real-time chain reaction (Real-time PCR) allows that the identification of microorganisms is more rapid, sensitive, and specific. This promises to be an alternative quantitative method to culture [10].

The present study was performed to compare the bactericidal effect of *Enterococcus faecalis* of 810 nm diode laser versus 3% sodium hypochlorite by culture method and qPCR.

Materials and methods

Sample: 34 upper and lower premolars have 1 root and 1 canal.
Recruiting method: Convenient sample without probability.

Recruiting criteria

For patients:
- Tooth extraction because of orthodontic treatment purpose and patients agreed with indications.
- The patient does not retain teeth after extraction.
- The patient's age is from 18 to 35 years old.

For teeth:
- Upper or lower premolars with 1 root.
- The crown of teeth is intact, never endodontic treatment, no fillings or cavities.
- Roots have no deep holes, no internal or external contents, no cracks or fractures.
- Root tips are mature.
- The canal is not calcified (the canals are examined with the K-file # 15 endodontic file).

Study process

After being extracted, teeth are washed blood and secretions under the tap water, then soaked in 2% Hexanios solution. Using ultrasonic liming machine cleans the periodontal ligament, tartar, alveolar bone ... After being cleaned, teeth are preserved in 0.9% NaCl solution until the test. Put the teeth in the set the root length from the tip to the neck, marked at the position of 13.0 mm. Use the diamond disc to cut in the marked position. Take marrow with thorns. Determine the working length for each canal, insert the K-file # 15 (Dentsply, Maillefer, Switzerland) into the canal until the instrument appears in the root opening. Then subtract 1,0 mm, recording the working length of each root. Preparing canals with ProTaper Universal hand file, all canals were prepared with SX, S1, S2, F1, F2, F3, F4 in turn. Use distilled water to pump and rinse during preparation. Each canal was then filled with lubricate (Glyde, Dentsply Sirona, Maillefer, Switzerland) for 3 minutes and then washed with 0.9% NaCl solution, followed by 3% Sodium Hypochlorite for 5 minutes. Each canal was further pumped with distilled water, then dried with a paper cone. Composite resin is used to fill the apical foramen. Continue to apply 2 coats of varnish outside the root, let dry for 24 hours. Each root is buried in Eppendorf plastic tube with rubber block. Then, all plastic tubes containing root teeth were autoclaved at 121^0 C for 20 minutes. Store in a sterile cabinet at 30^0 C, 100% humidity until use.

Hydrate the lyophilized bacteria *Enterococcus faecalis* ACTT 29212 according to the manufacturer's instructions, sweep onto prepared SF agar plates, then incubate in CO_2 incubators at 37° C for 48 hours. Activate bacteria by taking 1-2 pinch of bacteria inoculated in BHI broth at 37^o C for 6 hours. Adjust with BHI broth until the bacterial suspension is equivalent to McFarland No.1 (about 3x108 CFU / ml). The turbidity of the suspension was determined using the BD PhoenixSpec ™ turbidity meter. Groups including: negative control group, Group A: Randomly select 2 teeth in group., study group: 32 remaining teeth. Group A: use a semi-automatic micro-suction nozzle to pump 20µl of sterile BHI medium into each root canal. Experimental group: bacteria suspension was mixed well, using a micro-tip semi-automatic to pump 20µl into each canal. The K-file # 15 is inserted into the canal - with a rubber stopper at the right working length, bringing the file back and forth so that the BHI medium and bacteria are suspended to reach the instrument.

Utilising of sample was set at 37^0 C for 21 days (add sterile BHI medium every 48 hours). After 21 days of incubation at a temperature of 37^0 C, 2 teeth were randomly selected from a

group of 32 teeth for testing. Remove the teeth from the eppendorf, using a slow drill to cut 2 grooves on the Outer and Inner sides, along the root axis. Use a chisel to separate the teeth legs into 2 parts. Soak the teeth parts in formalin 10% solution for 7 days. Then wash the 70% - 80% - 90% - 95% - 100% - 100% ethanol solutions in turn, for 15 minutes each. Samples were gold coated and scanned using the Scanning Electronic Microscope (SEM, JOEM, Tokyo, Japan). Teeth slice samples were taken at 3 positions A, B, C corresponding to 1/3 of neck, middle 1/3 and 1/3 of tooth tip, magnification x2500 and x5000 (figure 2.6). Then position A was taken at magnifications x10000, x20000 and x30000 to examine the dentin surface of root canal.

After 21 days of infection, the experimental group was randomly selected into 3 groups and coded as follows:

Group B (control group): Randomly select 10 teeth into group.

Group C: (n = 10) irrigating with 2 ml of 3% Sodium Hypochlorite within 1 minute according to the procedure: 2 ml of 3% Sodium Hypochlorite solution is contained in a sterile 5ml syringe, 30 Gauge wash needle, using a needle new for each tooth. Place the rinsing syringe needle tightly in the canal, back 1 mm, then pump at 2 ml / min. Pumping time: 1 minute. Then inject 2 ml of Sodium Thiosulfate 0.1 M solution into the canal to neutralize the chlorine base, after 1 minute, flush with 2 ml of saline again.

Group D: (n = 10) laser diode 810nm on each canal according to the process: Activate the optical fiber end with dark paper. Insert the fiber tip into the canal to a working length of 1mm. Activating the laser, draws in a spiral direction at a speed of 2mm / sec. Activation time 7 seconds per cycle. Number of laser processing cycles: 5 cycles per tooth. Rest time between each cycle 20 seconds. Use a new fiber optic tip for each root canal.

Each tooth is determined by the number of bacteria in the canal.

Step 1: Using K - file # 25 bended the head, put into the canal to the working length, just rotate clockwise 3 turns while withdrawing, then all 3 groups of canals are filled with capacity. 0.9% NaCl solution is sterile.

Step 2: Put 3 sterile paper # 25 nubs in turn with the length corresponding to the working length into the canal. Each paper cone is left to rest for 1 minute and then removed, the next paper cone is inserted.

Step 3: The paper cone is transferred to a plastic tube containing 2ml of sterile physiological saline solution. Then solution containing 3 paper cones in plastic tube is mixed with Vortex machine for 1 minute.

Step 4: The samples are divided into 2 parts, 1ml per plastic tube. One tube for qPCR testing and one culture test tube.

Determination of bacteria by qPCR: The plastic tube for qPCR test was immediately taken to Nam Khoa Biotek Company and stored at -20OC until analysis. Quantitative results of *E. faecalis* will be returned by Nam Khoa Biotek company after 3-5 days.

Determining the number of bacteria by culture: Using a semi-automatic microbial tip, take 1ml of solution from a plastic tube for culture testing, continuously dilute 3 concentrations with group B and 1 concentration with group C, D. Then take 20µl of the diluted solution and spread it into a dish of SF medium. Place the agar plates that have spread bacteria in the incubator, the temperature 37^0 C. After 48 hours of determining the number of bacterial colonies, the number of bacteria's growth is determined by determining the number of bacteria growing on the SF medium plate.

Results

The general characteristics of the teeth of the subjects included in the present study were displayed in the Table 1.

	Position		Number of roots	Gender		Extraction reason
	Upper	Lower	1 root	men	woman	Orthodontic purpose
n	15	15	30	14	16	30
%	50	50	100	46,67	53,33	100

Table 1. General characteristics of study subjects (n = 30).

The average number of bacteria in the three experimental groups using the cultural method were displayed in the Table 2. There were significant differences among the three groups.

The average number of bacteria in the three experimental groups using the qPCR method were displayed in the Table 3. There were significant differences among the three groups.

	Average number of bacteria (x10⁴ CFU/ml)	Log number of bacteria (log10 CFU/ml)	T – test		
Group B	577 ± 142,24	6,75 ± 0,04	p=0,000		
Group C	1,91 ± 0,70	4,24 ± 0,06	<0,001	p=0,000	p=0,000
Group D	4,33 ± 0,56	4,63 ± 0,02		<0,001	<0,001

Table 2. Average number of bacteria of the 3 groups according to the cultural method.

	Average number of bacteria (x10⁴ CFU/ml)	Log number of bacteria (log10 CFU/ml)	T – test		
Group B	306 ± 57,81	9,48 ± 0,03	p=0,000		
Group C	1,72 ± 0,27	7,23 ± 0,02	<0,001	p=0,000	p=0,000
Group D	21,06 ± 10,35	8,28 ± 0,06		<0,001	<0,001

Table 3. Average number of bacteria (log transformation) of 3 groups according to qPCR method.

The average number of bacteria in the three experimental groups using both the cultural and the qPCR method were displayed in the Table 4. There were significant differences between the two methods of evaluation.

	Cultural	qPCR	t – test
Group B	6,75 ± 0,04	9,48 ± 0,03	p=0,000 < 0,001
Group C	4,24 ± 0,06	7,23 ± 0,02	p=0,000 < 0,001
Group D	4,63 ± 0,02	8,28 ± 0,06	p=0,000 < 0,001

Table 4. The difference in bacterial counts of groups according to culture method and qPCR.

Discussion

The experimental group using Sodium Hypochlorite solution (group C) had less bacteria than control group B (average was 4.24 in group C and 6.75 in group B). This difference is statistically significant and corresponds to a decrease in bacteria of about 99.69%. Group D (diode laser) had an average number of bacteria 2.12 log less than control group B (4.63 in group D and 6.75 in group B). This difference is also statistically significant and corresponds to a reduction in bacteria of about 99.24%. Specially, the average number of bacteria of group C was 0.39 log less than group D, although small but still significant (p <0.001). Thus, both SH solution and diode laser 810 nm are bactericidal, but the efficiency of SH solution (99.69%) is higher and statistically significant compared to 810 nm diode laser (99.24 %). This result is similar to the research's results of most other authors, when it is suggested that 810 nm diode laser is capable of killing *E. faecalis* but less than Sodium Hypochlorite solution.

The number of bacteria in group C (7.23 log10) and group D (8.28) was less than in group B (control group 9.48), in which group C was less than group B (corresponding level the reduction of bacteria was 99.44%), group D was less than that of group B (corresponding to reduction of bacteria was 93.69%) and these differences were statistically significant (p <0.001). Besides, group C was 1.05 log less than group D, statistically significant (7.23 versus 8.28). As a result, both Sodium Hypochlorite solution and diode laser are bactericidal, but the efficiency of 3% Sodium Hypochlorite solution (about 99.44%) is significantly higher than that of diode laser (about 93.69%). This conclusion is similar to the result from the cultural method.

The results of 2 cultural methods and real-time PCR have big difference. These results are similar to many studies in the world, most authors believe that the quantification of bacteria by qPCR is higher than quantitative by culture. This difference in results can be explained by many reasons: the first is the culture method which is complex, time consuming and many steps to perform, leading to the possibility of error to accumulate. The implementation process must be diluted many times, especially in the control group, the concentration of bacteria is no longer accurate, much dilution also makes a number of bacteria die, or go into VBNC state, increasing the error. In the experimental group, the biocides may remain in the sample, further killing or inhibiting the living bacteria, reducing the likelihood of cramping, resulting in lower culture results, even less. fake negative lice. Second, the sensitivity of PCR is much higher than in culture, most authors believe that PCR is detectable at 10-100 cells/ml, while culture requires a minimum concentration of 103 to 104 cells/ml. ml means that the culture will be inaccurate if the concentration of bacterial cells in the sample is below the threshold.

Currently, culture is still the gold standard in the diagnosis and quantification of microorganisms, as well as research on properties and prediction of pathogenesis using the colony counter or ELISA [11, 12]. Along with the culture, the modification of the root canal wall by diode laser is also investigated [13]. In operative dentistry, the diode laser is also evaluated for the microleakage reduction capability when

compared to other methods [14].

The new techniques based on molecular biotechnology, such as qPCR with outstanding advantages such as high sensitivity, low detection threshold, fast time ... are becoming a new trend in research and practice. qPCR is an adjunct to clinical and research cultures. With studies evaluating bactericidal efficiency, qPCR can be an alternative to traditional culture methods.

Conclusion

As the result collecting above, it can be concluded: Laser diode 810 nm is capable of killing *Enterococcus faecalis* bacteria in the canal. The bactericidal efficiency of the 810 nm diode laser for *E. faecalis* is less than that of the sodium Hypochlorite solution 3%. Real-time PCR can be used to complement culture methods in endodontic bactericidal efficacy studies.

Declaration of Interest

The authors report no conflict of interest.

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