

Original Article

Antibacterial effects of pulsed Er:YAG laser radiation at different energy settings in molars with root canal isthmuses

Meng-Qi Zhou¹, Hao-Ming Wang¹, Jia-Qi Xiao², Jin Hong¹

¹Shanghai Ninth People's Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai, China; ²School of Medicine, Shanghai Jiao Tong University, Shanghai, China

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Abstract: Er:YAG lasers have been demonstrated the usefulness for disinfection of anterior teeth with single root canal. However there has been few articles about the antibacterial effects in molars with root canal isthmuses (RCIs). 160 roots containing RCIs were selected, prepared up to file #40, and sterilized. They were divided evenly into two groups using randomization method, and inoculated with either *E. faecalis* or *E. coli*. The canals were either rinsed by 2% Chloramine T and 2% H₂O₂ (hydrogen peroxide) alternately for 30 s or irradiated with an Er:YAG laser for 30 s. The output power of Er:YAG laser was set as: 1.5 W, 2 W, 2.5 W, 3 W, 3.5 W, and 4 W respectively. The bacteria remaining after irradiation were counted using colony counting method. The results show that Er:YAG laser has better antibacterial effects than 2% Chloramine T and 2% H₂O₂ irrigation. Moreover, we confirm 2.5 W is the optimal output power of the Er:YAG laser to effectively kill both *E. faecalis* and *E. coli* in molars with RCIs. Additionally, the *E. coli* is more sensitive to Er:YAG laser than *E. faecalis* at low power setting (≤ 2 W).

Keywords: Er:YAG laser, root canal isthmus, *enterococcus faecalis*, *escherichia coli*

Introduction

The root canal isthmus (RCI) is one of the common anatomical complexities in root canal systems [1, 2]. According to the study of Estrela et al, the RCI frequency of mandibular first molars was as high as 87.9% [3], similar with the 83% from study of von Arx T et al. [4]. the existence of the RCI may limit the action of endodontic instruments, chemical irrigant solutions and intracanal medications, which are primary clinical approaches to eliminate microorganisms and the key to successful root canal therapy (RCT, a main treatment for endodontic disease) [5]. The residual pulpal tissue, micro-organisms and dentine debris may persist in the RCI and re-infect the root canal system, even the peripheral tissues, leading to the failure of RCT. The inability of the conventional approach to bacteria elimination has been one major cause for the failure of RCT. Thus, to improve the success rate of RCT, it is necessary to find a new method to sterilize molars with RCIs efficiently.

The Er:YAG laser was introduced into restorative dentistry in 1990 [6]. It has been demonstrated that the Er:YAG laser has a significant antibacterial effect in single root canal [7-14]. The effect produced by Er:YAG laser on tissues or organisms is due to photo-mechanical actions, photo-chemical, and photo-thermal disinfection. First of all, the Er:YAG laser with a wavelength of 2.94 μm corresponds to the maximum absorption peak of hydroxyapatite and water molecules. Since the major component of dentine from root canal wall is exactly hydroxyapatite, large amounts of energy can be absorbed by dentin in a short time and then causing 'micro-explosions' effect, which is similar to the effect of cutting. Then the dentine attached by bacterial film can be cut off [15, 16]. Secondly, the Er:YAG laser has the same effect as chemical disinfection. After the absorption of laser energy, the water molecules are separated under the counteraction of intermolecular force and the relaxation force to produce abundant, disinfecting oxygen free radicals. Gram-negative facultative and ana-

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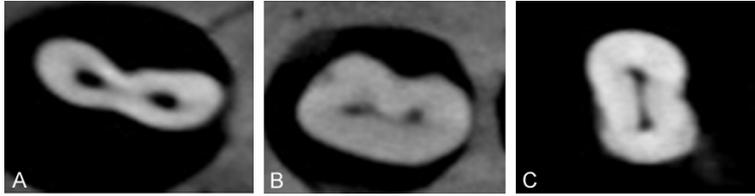


Figure 1. Cross-section image of selected roots containing RCIs scanning obtained by CBCT scanning. A: Type II (two root canals with a clear connection between the 2 main root canals). B: Type IV (Canals extending to the isthmus). C: Type V (a true connection or corridor throughout the section).

erobes bacteria, which are common bacteria in root canal system, are sensitive to the toxic effects of oxygen compounds [17]. In addition, the Er:YAG laser has well-known photo-thermal destructive effects on outer membrane of bacteria, thus directly killing the bacteria [28]. The risk of thermal damage can be reduced by operating the Er:YAG laser in pulsed modes [18].

Material and methods

Specimen preparation

Fully-extracted mandible first molars which contained two roots were collected. Cone-beam computed tomography (CBCT; Planmeca Pro-Max3Dmax operated at 96 kV, 10 mA, 12 s) at the First Dental Clinic of Shanghai Ninth People's Hospital was used for non-invasive CBCT images [19]. Each tooth was placed apical-coronally inside a custom-made holder. After scanned, roots with two canals and an isthmus between them were selected according to the criteria following: 1) complete root; 2) no endodontic treatment; and 3) the presence of root canal isthmus. According to the Hsu & Kim classification standard [20], root canal isthmus can be divided into five types. In this study, we selected Types II, IV and V (Figure 1A-C). Two roots from one molar were separated using a rotary diamond saw (NSK MACH-QD, Japan) at 700 rpm and the root without an isthmus was discarded. The entire procedure was performed by the same operator.

The selected roots were cleaned and sectioned to a constant root length of 14 mm using a rotary diamond saw (NSK MACH-QD, Japan) at 700 rpm. The canals were instrumented using a standardized crown-down method. The canal length was determined with the size 10 stainless steel hand files just visible in the

apical foramen of each canal. The working length was established 1 mm short of the canal length. The canals were enlarged to 40# (Ni-Ti root canal files, 06 taper, K3 System, TCM Endo III). Throughout the instrumentation procedure, the canals were rinsed with 2.5 ml of 5.25% NaOCl (sodium hypochlorite) and 2.5 ml of 2% H₂O₂ once a file was

exchanged (washed by sterile syringes, pinheads were placed at the root canal orifice but not clipped tightly). The shaped canals were finally rinsed with 2.5 ml NaCl (sodium chloride).

After re-scanned by CBCT, 160 roots with two canals and an isthmus were included in this study. All of these roots were then coated with nail polish and the apical region was sealed with resin to prevent bacterial leakage. The root specimens were all prepared by the same operator.

The prepared specimens were sterilized by autoclaving (121°C, 20 min) and dried in a drying oven. Then, the internal part of each root canal was dried by a sterile paper point, and all root specimens were placed in sterile test tubes until use.

Bacteria preparation

Enterococcus faecalis ATCC29212 (Institute of Microbiology, Chinese Academy of Sciences) and *Escherichia coli* ATCC25922 (Institute of Microbiology, Shanghai Jiao Tong University School of Medicine) were used.

ATCC29212 and ATCC25922 were diluted to approximately 3.0×10^8 colony-forming units per milliliter (CFU/mL) using McFarland's turbidimetry. Each bacterium was sampled, inoculated into a brain heart infusion (BHI, Oxoid CM1135B) agarose medium, and cultivated at 37°C under oxygen for 24 h. The purity and concentration of each bacterium were assessed.

Half of the roots were inoculated with 2.5 ml *E. faecalis* suspension and marked as group A, the other half were inoculated with 2.5 ml *E. coli* suspension and marked as group B. Roots were immersed into the bacterial suspension and the tubes were sealed with a rubber plug,

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Figure 2. Sealed test tubes with experimentally infected specimens in vacuum cell.



Figure 3. Er:YAG dental laser system (LITETOUCH, Syneron Medical Ltd, Yokneam, Israel).

enveloped with a rubber band and placed in vacuum for 30 min (**Figure 2**).

All tubes were placed in a constant-temperature incubator at 37°C and cultured for 2 weeks. At 2-day intervals, the BHI broth was randomly sampled from the tubes to monitor the purity of *E. faecalis* and *E. coli* (using the same method as mentioned above). Fresh BHI was exchanged every 3 days.



Figure 4. Specimens irradiated by Er:YAG laser at different powers. The whole procedure was performed in biosafety cabinet.

Both group A and group B were evenly divided into 8 subgroups using randomization method. Subgroups 1-6 were selected as experimental groups, subgroup 7 as a negative control group and subgroup 8 as positive control group.

Laser preparation

Er:YAG dental laser system (LITETOUCH, Syneron Medical Ltd, Yokneam, Israel) was used (**Figure 3**). The spray cooling device at the laser head was switched on, and sterile distilled water was used as the coolant.

Working tip with a diameter of 1.3×19 mm was used.

Specimen processing

Specimens were further divided into 7 subgroups from group *faecalis* and group *coli* of Group A respectively. The same procedure was performed for Group B. 1-6 subgroups were irradiated by Er:YAG laser at an output power of 1.5, 2, 2.5, 3, 3.5 and 4 W respectively. Before the irradiation, the canals were filled with sterile distilled water using sterile syringes. The laser head was placed at one root canal orifice and kept stationary for 15 s and the other orifice for another 15 s. The spray cooling device was able to prevent thermal-damage from temperature rising. The laser head was exchanged once the whole procedure for one specimen was accomplished (**Figure 4**).

Subgroups 7 were left untreated and subgroups 8 were rinsed with 2% Chloramine T and 2%

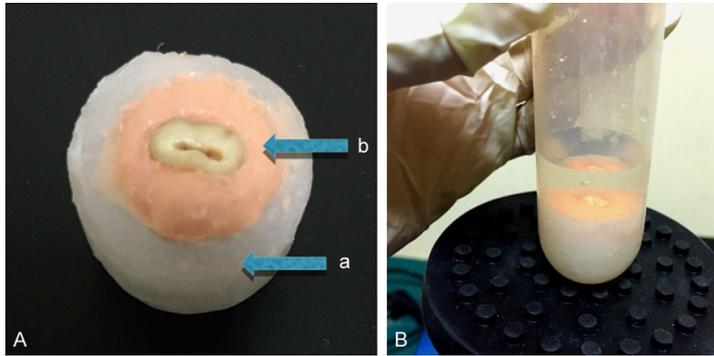


Figure 5. A: Custom-made holder. a: Acrylic b: Polyvinylsiloxane impression material; B: Specimens were embedded in custom-fabricated holder and put into the tube filled with 4 ml sterile saline. The tube was put on vortex machine for 1 min.

H₂O₂ alternately for 30 s at the speed of 3.5 ml/min.

A holder was made by the material of acrylic (Orthocryl, Dentaaurum GmbH&Co, Germany) and to the same diameter of the tube used for vortex. After irradiation, the surface of roots were coated with nail polish and a shallow groove was made as an anti-rotation lock. The acrylic holder was filled with polyvinylsiloxane (PVS) impression material and the root was imbedded in. Then the holder was placed into tube, then 4 ml sterile saline was infused into the tube. The tubes were placed in vacuum for 30 min. The nail polish and the holder can prevent bacteria attached on the root surface from mixing with bacteria from internal root canals during sampling procedure to make sure the outside bacteria can be excluded from bacteria counting (Figure 5A, 5B). After vortexing for 1 min, decimal dilutions were made to allow the number of colony-forming units of *E. faecalis* and *E. coli* to be determined. From the appropriate dilutions, aliquots of 0.5 ml were seeded in duplicate on Petri plates containing the BHI agar, and the plates were incubated at 37°C for 24 h. Then, the number of remaining bacteria was determined.

Statistical analysis

Statistical analysis was performed using the SPSS 18.0 package for windows. The data are expressed as the mean ± standard deviation (SD). Tukey's multiple comparison test was used for subgroups and One-way analysis of variance was used for Group A and Group B. *P* values <0.05 were considered to be significant.

Results

For subgroups 1-6, the numbers of remaining colonies (both *E. faecalis* and *E. coli*) all decreased significantly compared to the negative control groups (*P*<0.05), and showing a significantly better bactericidal effect than positive control groups (2% Chloramine-T and 2% H₂O₂). At 1.5 and 2 W, the bactericidal effects were not different significantly from one another, whereas at 2.5 W, the bactericidal effect increased significantly. The bactericidal

effects at 3, 3.5 and 4 W were similar to each other.

At ≤2 W, the Er:YAG laser irradiation had significantly better bactericidal effects to *Escherichia coli* than to *Enterococcus faecalis* (*P*<0.05). When the power ≥2.5 W, the bactericidal effects to these two species were similar (Table 1).

Discussion

Gram-positive *E. faecalis*, is detected in almost 1/3 of unsuccessful RCT cases [21]. It is resistant to several antimicrobial agents, and can survive thermal variations [12]. *Enterococcus faecalis* (ATCC29212) was selected as one of the experimental bacteria for this study because of its persistence, resistance and capacity to provide a long-term nidus for subsequent infection after RCT [22, 23].

Escherichia coli, as a standard organism used in antimicrobial testing, is sometimes recovered from root canals [24]. Nearly half of infected root canals include Gram-negative bacteria [25]. Thus, we selected *E. coli* (ATCC25922), representing Gram-negative bacteria, as one of the test microorganisms to compare the bactericidal effects of laser irradiation on Gram-positive and Gram-negative bacteria.

Bacteria exist in the form of a biofilm on the root canal wall. After adhering to the contact surfaces, bacteria form an aggregated, film-like structure that can resist destructive external forces [26]. The formation of bacterial biofilms

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Table 1. Bacterial counts and reduction percentage in the experimental and control groups

Power (W)	Group A (<i>Enterococcus faecalis</i>)		Group B (<i>Escherichia coli</i>)	
	Count (10 ³ CFU/mL)	Reduction (%)	Count (10 ³ CFU/mL)	Reduction (%)
1.5	42.550±72.033	98.255	39.125±11.880	98.634
2	41.770±57.603	98.287	32.150±13.300	98.878
2.5	13.075±7.903	99.464	12.300±5.287	99.570
3	12.920±8.295	99.470	11.900±6.266	99.584
3.5	12.490±11.241	99.487	11.500±6.887	99.598
4	10.941±6.638	99.551	10.825±4.681	99.622
0 (negative control)	2439±1048	0	2866.25±1923	0
2% Chloramine-T and 2% H ₂ O ₂ (positive control)	238.729±23.015	90.212	249.22±29.871	91.305

*The bacteria were irradiated with the laser at 1.5 W, 2 W, 2.5 W, 3W, 3.5 W, 4 W or rinsed by 2% Chloramine-T and 2% H₂O₂ for 30 s. The data are expressed as the mean ± SD. One-way analysis of variance was used to compare the two groups (i.e. the different bacteria).

takes time. Thus, we set the bacterial culture time at 2 weeks [27], which guarantees the formation of a sufficient bacterial biofilm on the root canal wall.

The presence of bubbles in the culture medium inhibits the adhesion of bacteria to the dentine wall of the isthmus which is a narrow and long structure. Thus, the inoculated specimens were placed in a vacuum environment for 30 min to remove all bubbles from the entire root canal system, including the isthmus, to ensure that the bacterial suspension can fully contact the isthmus.

The purpose of placement in vacuum after irradiation is to let saline into isthmus part in order to include the remaining bacteria in RCLs into counting. Counting in this new way can make sure that only remaining bacteria from internal root canals can drift with liquid out of root canal system and into the sterile saline in the test tube as much as possible. It is better than the way of dip with paper point.

In this study, the Er:YAG laser could be set to output powers of 1.5 W, 2 W, 2.5 W, 3 W, 3.5 W, or 4 W. Substantial reduction in CFU was obtained with both *E. faecalis* and *E. coli*. Although a higher antibacterial effect was achieved at higher laser power settings, the Er:YAG laser power and the antibacterial effect did not correlate in a simple, linear manner. The antibacterial effect at 2.5 W was significantly higher than that at lower power. Although the antibacterial effect was higher at a power setting larger than 2.5 W, the difference in bacterial reduction between them (bacteria exposed to 2.5 W, 3 W, 3.5 W and 4 W) was not statistically significant. Even at the maximum

laser power in this study (4 W), the Er:YAG laser did not result in complete eradication of the bacteria. This result is essentially consistent with previous studies [28, 29]. Thus, when the Er:YAG laser is used to sterilize root canals with an isthmus, the lowest power that can yield the highest antibacterial rate should be selected. This will avoid heat damage to periodontal tissues resulting from the use of excessive power.

At ≤2 W, the antibacterial effect of the laser on *E. coli* was significantly higher than that on *E. faecalis*. That is to say the *E. coli* was more sensitive to Er:YAG laser than *E. faecalis* at low power settings (≤2 W).

In conclusion, the Er:YAG laser has better antibacterial effects than 2% Chloramine-T and 2% H₂O₂ irrigation. More importantly, 2.5 W has been determined to be the optimal output power to effectively kill both *E. faecalis* and *E. coli* in molars with root canal isthmuses. The application of Er:YAG laser to root canal disinfection is very useful for molars with root canal isthmuses.

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Disclosure of conflict of interest

None.

Address correspondence to: Dr. Jin Hong, School of Medicine, Shanghai Jiao Tong University, Shanghai Ninth People's Hospital, The First Dental

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Department, No. 639, Zhi Zao Ju Road, Shanghai, China. Tel: 0086-21-53078098; Fax: 0086-21-63133174; E-mail: hongjin1221@yahoo.com

References

- [1] Weller RN, Niemczyk SP and Kim S. Incidence and position of the canal isthmus. Part 1. Mesiobuccal root of the maxillary first molar. *J Endod* 1995; 21: 380-383.
- [2] Teixeira FB, Sano CL, Gomes BP, Zaia AA, Ferraz CC and Souza-Filho FJ. A preliminary in vitro study of the incidence and position of the root canal isthmus in maxillary and mandibular first molars. *Int Endod J* 2003; 36: 276-280.
- [3] Estrela C, Rabelo LE, de Souza JB, Alencar AH, Estrela CR, Sousa Neto MD and Pecora JD. Frequency of root canal isthmi in human permanent teeth determined by cone-beam. *J Endod* 2015; 41: 1535-1539.
- [4] Von Arx T. Frequency and type of canal isthmuses in first molars detected by endoscopic inspection during periradicular surgery. *Int Endodont J* 2005; 38: 160-168.
- [5] Lima FJ, Montagner F, Jacinto RC, Ambrosano GM, Gomes BP. An in vitro assessment of type, position and incidence of isthmus in human permanent molars. *J Appl Oral Sci* 2014; 22: 274-281.
- [6] Fornaini C. Er:YAG and adhesion in conservative dentistry: clinical overview. *Laser Ther* 2013; 22: 31-35.
- [7] Noiri Y, Katsumoto T, Azakami H and Ebisu S. Effects of Er:YAG laser irradiation on biofilm-forming bacteria associated with endodontic pathogens in vitro. *J Endod* 2008; 34: 826-829.
- [8] Stabholz A, Sahar-Helft S and Moshonov J. The use of lasers for cleaning and disinfecting of the root canal system. *Alpha Omegan* 2008; 101: 195-201.
- [9] Yasuda Y, Kawamorita T, Yamaguchi H and Saito T. Bactericidal effect of Nd:YAG and Er:YAG lasers in experimentally infected curved root canals. *Photomed Laser Surg* 2010; 28 Suppl 2: S75-78.
- [10] Cheng X, Guan S, Lu H, Zhao C, Chen X, Li N, Bai Q, Tian Y and Yu Q. Evaluation of the bactericidal effect of Nd:YAG, Er:YAG, Er,Cr:YSGG laser radiation, and antimicrobial photodynamic therapy (aPDT) in experimentally infected root canals. *Lasers Surg Med* 2012; 44: 824-831.
- [11] Meire MA, Coenye T, Nelis HJ and De Moor RJ. Evaluation of Nd:YAG and Er:YAG irradiation, antibacterial photodynamic therapy and sodium hypochlorite treatment on *Enterococcus faecalis* biofilms. *Int Endod J* 2012; 45: 482-491.
- [12] Dos Santos Antonio MP, Moura-Netto C, Camargo SE, Davidowicz H, Marques MM, Maranhão de Moura AA. Bactericidal effects of two parameters of Er:YAG laser intracanal irradiation: ex-vivo study. *Lasers Med Sci* 2012; 27: 1165-1168.
- [13] Tosun E, Tasar F, Strauss R, Kivanc DG and Ungor C. Comparative evaluation of antimicrobial effects of Er:YAG, diode, and CO(2) lasers on titanium discs: an experimental study. *J Oral Maxillofac Surg* 2012; 70: 1064-1069.
- [14] Sadik B, Arikan S, Belduz N, Yasa Y, Karasoy D and Cehreli M. Effects of laser treatment on endodontic pathogen *enterococcus faecalis*: a systematic review. *Photomed Laser Surg* 2013; 31: 192-200.
- [15] Dube A, Bansal H and Gupta PK. Modulation of macrophage structure and function by low level He-Ne laser irradiation. *Photochem Photobiol Sci* 2003; 2: 851-855.
- [16] Lubart R, Kesler G, Lavie R and Friedmann H. Er:YAG laser promotes gingival wound repair by photo-dissociating water molecules. *Photomed Laser Surg* 2005; 23: 369-372.
- [17] Hauser-Gerspach I, Mauth C, Waltimo T, Meyer J and Stubinger S. Effects of Er:YAG laser on bacteria associated with titanium surfaces and cellular response in vitro. *Lasers Med Sci* 2014; 29: 1329-37.
- [18] Walsh LJ. The current status of low level laser therapy in dentistry. Part 2. Hard tissue. *Aust Dent J* 1997; 42: 302-306.
- [19] Pecora JD, Estrela C, Bueno MR, Porto OC, Alencar AH, Sousa-Neto MD and Estrela CR. Detection of root canal isthmuses in molars by map-reading dynamic using CBCT images. *Braz Dent J* 2013; 24: 569-574.
- [20] Hsu YY and Kim S. The resected root surface. The issue of canal isthmuses. *Dent Clin North Am* 1997; 41: 529-540.
- [21] Licata ME, Albanese A, Campisi G, Geraci DM, Russo R, Gallina G. Effectiveness of a new method of disinfecting the root canal, using Er, Cr:YSGG laser to kill *Enterococcus faecalis* in an infected tooth model. *Lasers Med Sci* 2015; 30: 707-12.
- [22] Noda M, Komatsu H, Inoue S and Sano H. Antibiotic susceptibility of bacteria detected from the root canal exudate of persistent apical periodontitis. *J Endod* 2000; 26: 221-224.
- [23] Sedgley CM, Lennan SL and Appelbe OK. Survival of *Enterococcus faecalis* in root canals ex vivo. *Int Endod J* 2005; 38: 735-742.
- [24] Basrani B, Tjaderhane L, Santos JM, Pascon E, Grad H, Lawrence HP and Friedman S. Efficacy of chlorhexidine- and calcium hydroxide-containing medicaments against *Enterococcus*

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- faecalis in vitro. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2003; 96: 618-624.
- [25] Schoop U, Kluger W, Moritz A, Nedjelic N, Georgopoulos A and Sperr W. Bactericidal effect of different laser systems in the deep layers of dentin. Lasers Surg Med 2004; 35: 111-116.
- [26] Flemming HC, Neu TR and Wozniak DJ. The EPS matrix: the "house of biofilm cells". J Bacteriol 2007; 189: 7945-7947.
- [27] Neelakantan P, Cheng CQ, Mohanraj R, Sriraman P, Subbarao C and Sharma S. Antibiofilm activity of three irrigation protocols activated by ultrasonic, diode laser or Er:YAG laser in vitro. Int Endod J 2015; 48: 602-10.
- [28] Mehl A, Folwaczny M, Haffner C, Hickel R. Bactericidal effects of 2.94 μm Er:YAG-laser radiation in dental root canals. J Endod 1999; 25: 490-493.
- [29] Vezzani MS, Pietro R, Silva-Sousa YT, Brugnera-Junior A and Sousa-Neto MD. Disinfection of root canals using Er:YAG laser at different frequencies. Photomed Laser Surg 2006; 24: 499-502.