



# Effect of a 940 nm Diode Laser and MTAD on the Decontamination of Root Canals With *Enterococcus faecalis*

Alvaro Tapia Gálvez<sup>1\*</sup>, Abel Teves Cordova<sup>2</sup>, Oniel Elías Juárez Vilcapuma<sup>3</sup>, Katherine Joselyn Atoche-Socola<sup>4</sup>, Sergio Jiménez Sánchez<sup>5</sup>

<sup>1</sup>School of Dentistry, Universidad Científica Del Sur, Lima Perú

<sup>2</sup>Department of Operative Dentistry, Endodontics and Dental Materials, Bauru School of Dentistry, University of São Paulo, Bauru, SP, Brazil

<sup>3</sup>Faculty of Biological Sciences, Universidad Nacional Mayor de San Marcos, Lima, Perú

<sup>4</sup>Division of Oral Rehabilitation, School of Dentistry, Universidad Científica Del Sur, Lima, Perú

<sup>5</sup>Division of Laser in Dentistry, School of Dentistry, Universidad Científica Del Sur, Lima, Perú

**\*Correspondence to**

Alvaro Tapia Gálvez,  
Email: 100018497@cientifica.edu.pe

**Received:** May 29, 2024

**Accepted:** September 15, 2024

**Published:** November 25, 2024



## Abstract

**Introduction:** In recent years, various methods such as lasers and different solutions have been investigated to eliminate microorganisms in the root canal. This investigation was aimed at comparing the bactericidal effect on *Enterococcus faecalis* between a diode laser with a wavelength of 940 nm and a mixture of a tetracycline isomer, an acid, and a detergent (MTAD). **Methods:** Forty single-rooted human lower premolar teeth were prepared for inoculation with *E. faecalis* and incubated for a period of 3 weeks. The 40 samples were randomly divided into 4 groups: the 940 nm diode laser group with irradiation through a 200 µm optical fiber with parameters of (1 W/CW, 4 cycles, 20s interval); the MTAD group irrigated with 5 mL for 5 minutes; also, a positive group with 3% NaOCl and a negative group with 0.9% saline solution, both irrigated with 5 mL for 5 minutes. For statistical analysis, the Mann-Whitney U and Wilcoxon tests were used.

**Results:** The 3% NaOCl positive control group had greater bacterial reduction after treatment (99.99%) compared to the 940nm diode laser (98.96%) and MTAD (99.34%) groups. Statistically, we found a significant difference between all groups regarding the reduction in the colony count ( $P < 0.05$ ).

**Conclusion:** It was found that there was a significant difference in the percentage of reduction for the bacterial colonies between all the groups evaluated, with the greatest antibacterial effectiveness observed with 3% sodium hypochlorite.

**Keywords:** Diode laser; *Enterococcus faecalis*; MTAD; Root canal therapy.

## Introduction

One of the main goals of endodontic treatment is to properly remove organic and inorganic debris lodged in the canal walls, and microorganisms inside the canal system. The main cause of endodontic failure is the presence of *Enterococcus faecalis* due to its resistance and ability to cause endodontic infection.<sup>1,2</sup>

To avoid endodontic failure, chemical substances with antibacterial capacity are used during instrumentation to enhance the proper rinse and disinfection of the root canal.<sup>3,4</sup> However, the root morphology renders root canal cleaning extremely challenging.<sup>5,6</sup> Therefore, in recent years new methods have been investigated. The diode laser (DLs) has shown great antibacterial capacity in endodontics for the decontamination of root canals. Furthermore, the diode laser can be used with different wavelengths through optical fibers that allow interaction

with the deeper layers of the dentin tubules.<sup>7-9</sup> The DLs can be used in continuous or pulsed mode through a flexible optical fiber conductor, penetrating the dentin tubules and eliminating microorganisms.<sup>10-12</sup> Another positive aspect of the diode laser is its versatility, compactness, portable size, and safety for clinical use.<sup>13</sup>

A wide range of solutions such as NaOCl, ethylenediaminetetraacetic acid (EDTA), chlorhexidine (CHX), potassium iodide and iodine are used for root canal disinfection. However, none of these irrigants meet all the requirements for endodontic treatment.<sup>14</sup> In endodontics, the most widely used solution is NaOCl, which has solvent, antibacterial capacity and is available at different concentrations. Nonetheless, it has disadvantages since it has a low penetration depth into the dentinal tubules and can induce injury to periapical tissues if it extravasates. Additionally, it is cytotoxic and has an unpleasant odor

and taste.<sup>15</sup> Given the limitations of hypochlorite, a new mixture of doxycycline, citric acid and a detergent (Tween 80) (MTAD) irrigant, manufactured in the United States in 2003, has been introduced to cover the requirements of endodontic treatment. It has excellent antibacterial properties and the potential to remove the smear layer from dentin. Besides, it has been shown to be effective and biocompatible with antimicrobial substantivity<sup>16,17</sup> To achieve greater antimicrobial success and to determine which therapy has greater efficacy, we compared the effect of 940 nm DLs and MTAD as co-adjuvants in endodontic treatment.

The use of diode laser and MTAD has not been widely studied, leading to a lack of research data. Therefore, the purpose of the present research was to contrast therapies in human single-root lower premolar teeth infected with *E. faecalis* American type culture collection (ATCC) 19433 evaluated by colony-forming units (CFU/mL) before and immediately after the application of the different treatments. It was hypothesized that MTAD and the 940 nm DLs have a similar ability to 3% NaOCl to decontaminate and obtain a significant reduction in the CFU/mL count in root canals infected with this microorganism.

## Materials and Methods

The sample and procedures performed were approved by the Ethics Committee of the Científica del Sur University.

### Sample Calculation

The sample calculation was performed using the G\*Power statistical software version 3.1.9.7 for Mac (University of Düsseldorf, Düsseldorf, Germany) by selecting the F-test family. In the present research, the effect size was established ( $= 1.42$ ). The alpha type error of 0.05 and a beta power of 0.95 were also stipulated. To observe significant differences, we designated nine samples in each group to obtain an ideal sample size. To compensate for possible losses, we added 10% of the samples. Consequently, a total of 10 samples were used for each group.

### Sample Preparation

Forty single-rooted premolar teeth extracted for orthodontic purposes were used. Then, they were stored, preserved and hydrated in sterile distilled water for a period of 3 months. They were confirmed to be single-rooted teeth through periapical radiographs. A cross-section was made, obtaining segments 14 mm from the apex with a metal disk at the level of the cement-enamel junction. The working length was 1 mm below the apical foramen. The canals were permeabilized through special #10 files until reaching master file #40 (Dentsply Maillefer, Ballaigues, Switzerland). Subsequently, they were instrumented with ProTaper Next X1-X2-X3 rotary instruments (Dentsply Maillefer, Ballaigues, Switzerland), irrigating the root

canals with 3% hypochlorite between rotary files. After the instrumentation, the canals were rinsed using 1 mL of 17% EDTA (Maquira, Maringá, Parana, Brazil), 5 mL of saline solution, and 1 mL of 2.5% NaOCl, respectively, each for 3 minutes by a 5 mL syringe with a Navitip cannula system (Ultradent, South Jordan, Utah, United States) of 0.30 mm in diameter to remove the smear layer. Then, they were irrigated using 5 mL of saline solution. Afterward, a self-curing glass ionomer (GC Gold Label, Tokyo, Japan) was used to seal the foramen. Furthermore, two coats of nail polish were used to cover the remaining root surfaces and allowed to dry for one hour. The 40 samples used in the study were sterilized before inoculation with *E. faecalis*. For this, the samples were placed in microtubes and sealed for sterilization in an autoclave for 30 minutes at a pressure of 15 psi at 121 °C.<sup>18</sup>

### Inoculation of Bacteria With *Enterococcus faecalis*

Standard strains of *E. faecalis* (American Type Culture Collection 19433) were certified by the laboratory (Microbiologics – Gen lab, Lima, Lima, Peru). Furthermore, they were cultured in 5 mL brain-heart infusion (BHI) broth 37 g/1 L (HiMedia laboratories, Mumbai, Maharashtra, India) and incubated at 37 °C for 24 hours. Each canal was inoculated using 0.1 mL of each BHI containing approximately a concentration of  $10^8$  *E. faecalis* bacteria by using a formula for calculating the number of microorganisms present in a test sample of two successive dilutions. Then, the samples were incubated at 37 °C for 3 weeks to form a biofilm. Besides, every 2 days the root canal was re-inoculated with 0.1 mL BHI containing 24-hour culture of *E. faecalis*.<sup>11,17,18</sup> The microbiological tests were carried out under conditions of sterility of materials and with a Bunsen burner, which provides a safety margin by forming a sterility area of approximately 15 to 25 cm in radius. The procedure was performed in an analysis area disinfected with 1% NaOCl and supplemented with 255 nm ultraviolet light before starting the analysis.

### Experimental Procedure

Negative control group: 10 randomly selected teeth were irrigated with 5 mL of saline solution for 5 minutes and the CFU/mL was evaluated before and immediately after the treatment in the root canals.

Positive control group: 10 randomly selected teeth were irrigated with 5 mL of 3% NaOCl for 5 minutes and then the CFU/mL was evaluated before and immediately after the treatment in the root canals.

Experimental group: 10 randomly selected teeth were irrigated with 5 mL of MTAD (Dentsply, Tulsa, Oklahoma, United States) for 5 minutes, and the CFU/mL was evaluated before and immediately after the treatment in the root canals.

Experimental group: 10 randomly selected teeth were irradiated with a 940 nm DL (Epic 10 Biolase, Irvine,

California, United States) in a helical apico-coronal direction for 4 cycles of 8 seconds each, with a 20-second interval between irradiations and with a power of 1 Watt/continuous wave (1W/CW) at 1 mm of the working length, using a flexible fiber of 200- $\mu$ m-diameter. Then, the CFU/mL was evaluated before and immediately after the treatment in the root canals.

### Microbiological Procedure

The samples were obtained before and immediately after applying the different treatments.

**Initial sample:** A reference microbial sample was obtained before the application of the treatments. A sterile #40 master file was used, and dentin chips were obtained by scraping for 20 seconds in the apical third and coronal area of the canal. Sterile #40 paper cones were placed inside the canal for one minute and then transferred to sterile micro-tubes containing peptone water. The samples from the paper cones in test tubes were homogenized in a vortex (INC, Zapopan, Jalisco, Mexico) for 10 seconds. Seven decimal dilutions of the sample were then made with 0.1% peptone water. From the diluted sample in the 10 mL test tube, an aliquot of 1 mL was placed in Petri dishes and then BHI agar tempered at 45 °C was deposited. The BHI agar was then homogenized with the inoculum of the corresponding dilution of the sample analyzed inside the plate. Petri dishes with BHI agar were allowed to cool for 15 minutes under sterile conditions in the seeding room and then incubated for 72 hours at 37 °C. After 72 hours of incubation, the CFU/mL of *E. faecalis* ATCC 19433 was counted in a digital colony counter (Kert-lab, Lima, Lima, Peru) with a dark background with magnifying glasses and with lighting under the Petri dish.

Subsequently, the *E. faecalis* colonies in the Petri dish were confirmed with BHI agar using the Gram staining technique. Therefore, a sowing loop with nichrome wire was used and the colony to be confirmed was taken from BHI agar. The colony of *E. faecalis* to be confirmed was transferred to a slide to perform with crystal violet staining for 1 minute, distilled water for 10 seconds, fixation with Lugol for one minute, distilled water for 10 seconds, acetone alcohol decolorizer for 15 seconds, water distilled water for 10 seconds, fuchsin for one minute, and distilled

water for 10 seconds. Then, the colored sheet was dried for a few minutes with the Bunsen burner. Furthermore, a drop of immersion oil was placed on the colored sheet to visualize Gram-positive cocci at 1000x magnification, thereby confirming the presence of *E. faecalis* in the extracted colony.

### Statistical Analysis

For the statistical analysis of the data, the SPSS version 27 program was used. The median and range of the CFU/mL values for each study group were calculated. The Shapiro-Wilk test was used to perform a normality analysis, and it was revealed that the data had a normal distribution. The homogeneity of variance test of the data was performed, and it was found that they did not have this characteristic, so nonparametric statistical tests were used to evaluate the results. Then, the data were analyzed by using the Wilcoxon test to establish whether there were significant differences between before and after the application of the treatments. To determine the most effective technique to reduce CFU/mL, the Mann-Whitney test was used. The calculation of the percentage reduction in the colony count (%RCC) according to CFU/mL was carried out by using the following equation:

$$\frac{\text{CFU}(\text{before treatment}) - \text{CFU}(\text{after treatment})}{\text{CFU}(\text{before treatment})} \times 100 = \%RCC$$

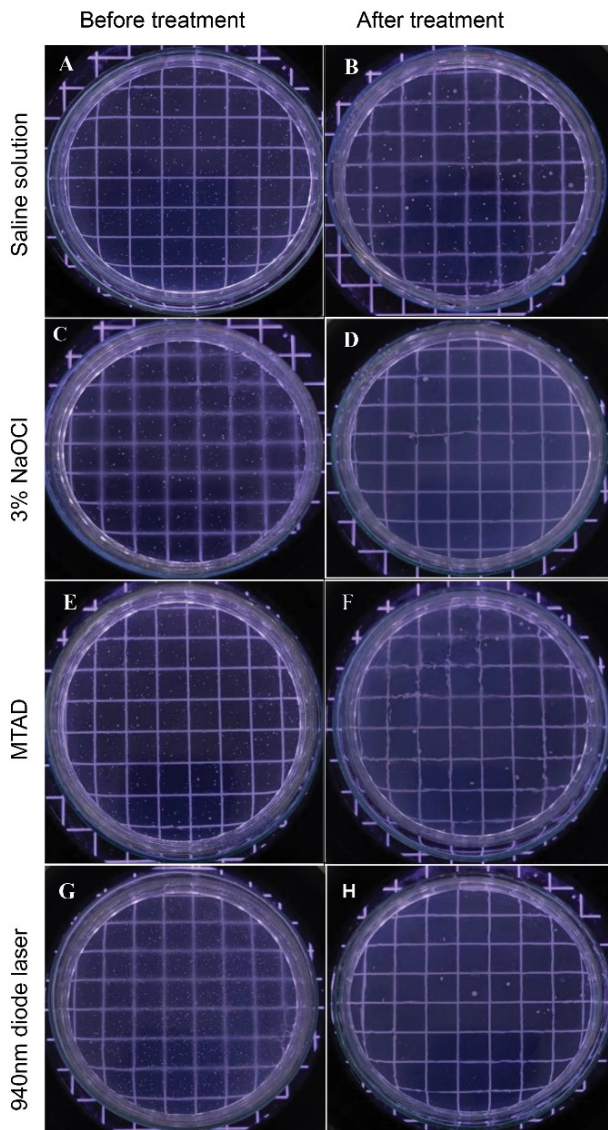
### Results

All groups showed bacterial growth after the inoculation of ATCC 19433 bacteria into the root canals.

Descriptive statistics were performed on the results in CFU/mL. In addition, the median, range and p-value were analyzed before and after each treatment performed (Table 1). The % RCC is shown based on the median of the counts before and after treatment (Figure 1 and Table 2). The Z-score test was performed, indicating that there was no existence of scattered data. Then, the Shapiro-Wilk test was applied to evaluate the normality of the data ( $n < 50$ ) for the values in CFU/mL for the microbial populations after the treatments with saline solution ( $P=0.564$ ), 3% NaOCl ( $P=0.732$ ), MTAD ( $P=0.550$ ) and the 940 nm diode laser ( $P=0.507$ ), and it presented normal

**Table 1.** Median, Range and P Values in CFU/mL of *Enterococcus faecalis* Before and After each treatment

Experimental Groups	Treatment	Median	Range	P Value (Wilcoxon Test)
Saline solution	Before	$7.10 \times 10^6$	$6.5 \times 10^6$ to $8.1 \times 10^6$	0.004
	After	$6.95 \times 10^6$	$6.2 \times 10^6$ to $7.9 \times 10^6$	
3% NaOCl	Before	$7.20 \times 10^6$	$1.10 \times 10^2$	0.005
	After	$6.2 \times 10^6$ to $8.0 \times 10^6$	$2.0 \times 10^4$ to $2.6 \times 10^2$	
MTAD	Before	$7.15 \times 10^6$	$4.65 \times 10^4$	0.005
	After	$6.4 \times 10^6$ to $8.2 \times 10^6$	$2.2 \times 10^4$ to $6.0 \times 10^4$	
940 nm diode laser	Before	$8.05 \times 10^6$	$8.35 \times 10^4$	0.005
	After	$6.8 \times 10^6$ to $9.1 \times 10^6$	$6.6 \times 10^4$ to $1.17 \times 10^5$	



**Figure 1.** *Enterococcus faecalis* ATCC 19433 Colony-Forming Units Before and Immediately After Therapies. \*Representative images of *Enterococcus faecalis* culture showing bacterial reduction before (left) and after (right) treatments: Saline solution (A-B), 3% NaOCl (C-D), MTAD (E-F), 940 nm diode laser (G-H)

distribution. Furthermore, the results did not present homogeneity of variances ( $P < 0.05$ ). Therefore, the non-parametric Wilcoxon test was used to establish that there were significant differences between before and after each treatment of the 4 experimental groups ( $P < 0.05$ ). The Mann-Whitney U test was performed to determine the most effective technique to reduce CFU/mL, from which the following was obtained: NaOCl 3% versus MTAD ( $P = 0.000$ ), 3% NaOCl compared to 940nm DL ( $P = 0.000$ ), and 3% NaOCl compared to saline ( $P = 0.000$ ). Lastly, 3% NaOCl is the most effective treatment against the 940nm diode laser and MTAD; however, the last ones can be used as co-adjuvants. (Table 3).

**Discussion**

The search for success in endodontics has led to the use of

**Table 2.** % RCC Values of the Different Groups

Group	RCC%
Saline solution	2.11%
3% NaOCl	99.99%
MTAD	99.34%
940 nm diode laser	98.96%

**Table 3.** Mann-Whitney U Test for Pair Comparison of the Groups

Treatment Comparison	P Value
3% NaOCl – Saline solution	0.000
3% NaOCl – MTAD	0.000
3% NaOCl – 940 nm diode laser	0.000

various disinfection methods to achieve the elimination of microorganisms in the canal system. The present study used a 940 nm diode laser due to its ability to disinfect root canals and reach deeper layers of dentin.<sup>9</sup> One modification was to incorporate the MTAD for its bactericidal properties and being less cytotoxic. For the evaluation of both treatments, clinical protocols were used to obtain reliable results.

According to Dai et al, *E. faecalis* is a gram-positive, facultative anaerobic bacterium that can be commonly isolated in failed endodontic treatments. An additional alternative to traditional root canal treatment would be the use of a 940 nm diode laser due to its antibacterial capacity.<sup>1</sup>

The diode laser delivery mechanism is through its flexible optical fiber ranging from 200 to 300  $\mu\text{m}$ , which can be applied in root canal treatment. Its radiation produces a bactericidal photothermal disrupting effect with the ability to destroy the bacterial cell wall.<sup>12,13,19,20</sup> According to this study, the lowest CFU/ml reduction was achieved by using a 940 nm diode laser. On the other hand, Schulte et al. used a similar protocol to compare the efficacy of a 940 nm diode laser where they measured the temperature changes in bovine teeth through dentin sections. They used two different laser systems, a radial firing tip (RFT) and a bare-end fiber tip (BFT). The most satisfactory result was obtained with the radial firing tip, obtaining a 99.99% reduction of CFU using a power of 1.5 W.<sup>21</sup> However, Castelo-Baz et al used a power of 3.5 W and reported a 70% bacterial reduction, where the samples were irradiated for one minute in total. This may be due to the use of a different microbiological protocol, shorter inoculation time of the bacteria, and a different way of applying the diode laser, which may affect the results.<sup>22</sup>

In the current study, a lower bacterial count was obtained by using a 940 nm diode laser when compared to 3% sodium hypochlorite. However, Afkhami et al. used an 810 nm diode laser with a power equal to the present study, where they reported a greater decrease of 97.41% compared to 2.5% NaOCl (%RCC 94.61%). This may be because they used a longer exposure time of the diode laser

with a rotary motion of 10 seconds, 4 times with 15-second intervals, and it was compared to a lower concentration of sodium hypochlorite.<sup>18</sup> In a research study, Shirani et al. compared the antimicrobial effectiveness of the 810 and 980 nm diode lasers, where they obtained a higher antibacterial effectiveness compared to 5.25% sodium hypochlorite.<sup>23</sup> One of the reasons why the diode laser had a greater positive effect on *E. faecalis* is due to its greater penetration capacity in the dentinal tubules as opposed to the conventional solution. In addition, the small absorption coefficient of this wavelength in water allows for greater penetration of the radiation, which may be another reason for achieving a better antibacterial effect.

Fahim et al evaluated an *in vivo* study where they used a combination of a 940 nm diode laser with EDTA. They obtained a reduction of 99.94% in anaerobic bacteria, and the treatment was performed in a single session. In contrast, this study used only a 940 nm diode laser for *in vitro* studies. However, similar results were obtained. Other investigated studies concluded that the combination of a diode laser with a solution enhances the effectiveness for the disinfection of root canals.<sup>12,22,24</sup>

According to other studies, MTAD contains citric acid and tween 80, which helps to remove the smear layer. It also contains doxycycline in powder form, which penetrates deeper into the dentinal tubules and exerts its antibacterial effect.<sup>25-27</sup>

In the present research, 3% NaOCl showed significant antimicrobial effectiveness against *E. faecalis*, where it was higher than MTAD. This contrasts with a study by Dubey, who used a lower concentration of 2.5% NaOCl.<sup>25</sup> Differences in results may be due to the use of different concentrations of sodium hypochlorite, variation in the strains tested, incubation time of the bacteria, as well as differences between the clinical protocols used.

Our study showed that the MTAD and 3% NaOCl groups showed significant differences between them. Priya et al. also obtained similar results, evaluating the reduction in CFU/ml between 1.3% NaOCl and MTAD combined with 1.3% NaOCl.<sup>28</sup> The similarity of the results can be attributed to the technique of sampling the canal content immediately after debridement. In addition, the same bacterial strain was used with a similar incubation time of *E. faecalis*. In another study, Nara et al evaluated 3% NaOCl and MTAD, and they found a statistically significant difference between both groups ( $P < 0.05$ ). The bacterial growth was determined by visualizing individual colonies of white dots on agar plates, unlike this study that determined the CFU/mL on Petri dishes that were confirmed with BHI agar, through the gram staining technique.<sup>27</sup>

In agreement with the present study, Ashofteh et al demonstrated in their investigation that there was a significant difference between the MTAD and an 830 nm diode laser ( $P > 0.004$ ). They used the diode laser in

circumferential motion with a higher power of 1.5 W and the same clinical protocol for the MTAD. However, they obtained the highest effectiveness with 5.25% NaOCl.<sup>7</sup>

Therefore, it can be argued that *E. faecalis* is the most frequent bacteria in endodontic failure. However, there are other bacteria present in the oral microbiota where the treatments used can be applied. Another approach that could contribute to bacterial decontamination is the use of different wavelengths of the diode laser to perform *in vivo* studies and apply them clinically.

## Conclusion

A comparison of the effect of a 940 nm diode laser and MTAD demonstrated that both have a high capacity for the elimination of *E. faecalis* in root canals when evaluated immediately after treatment. However, there was a significant difference when they were compared to 3% NaOCl.

## Acknowledgments

We thank all the co-authors of this study for their contribution to the writing, the support of the teams, and the analysis of the data. We also thank the Científica del Sur University for the material support and technical help.

## Authors' Contribution

**Data curation:** Alvaro Tapia Gálvez, Oniel Elías Juárez Vilcapuma, Sergio Jiménez Sánchez.

**Formal analysis:** Oniel Elías Juárez Vilcapuma, Abel Teves Cordova, Katherine Joselyn Atoche-Socola.

**Investigation:** All authors.

**Methodology:** Sergio Jiménez Sánchez, Katherine Joselyn Atoche-Socola, Oniel Elías Juárez Vilcapuma, Alvaro Tapia Gálvez.

**Project administration:** Sergio Jiménez Sánchez, Abel Teves Cordova.

**Resources:** All authors.

**Software:** Abel Teves Cordova, Oniel Elías Juárez Vilcapuma.

**Supervision:** Abel Teves Cordova, Katherine Joselyn Atoche-Socola.

**Validation:** Katherine Joselyn Atoche-Socola.

**Visualization:** Katherine Atoche, Abel Teves Cordova.

**Writing-original draft:** Alvaro Tapia Gálvez, Sergio Jiménez Sánchez, Oniel Elías Juárez Vilcapuma.

**Writing-review & editing:** Abel Teves Cordova, Katherine Joselyn Atoche-Socola.

## Competing Interests

None declared.

## Ethical Approval

This study received ethical approval from the ethics committee of Científica del Sur University (Code: 777-2020-PRE8).

## Funding

None.

## References

1. Dai S, Xiao G, Dong N, Liu F, He S, Guo Q. Bactericidal effect of a diode laser on *Enterococcus faecalis* in human primary teeth-an *in vitro* study. BMC Oral Health. 2018;18(1):154. doi: 10.1186/s12903-018-0611-6.
2. Prada I, Micó-Muñoz P, Giner-Lluesma T, Micó-Martínez P, Collado-Castellano N, Manzano-Saiz A. Influence of microbiology on endodontic failure. Literature review. Med

- Oral Patol Oral Cir Bucal. 2019;24(3):e364-72. doi: [10.4317/medoral.22907](https://doi.org/10.4317/medoral.22907).
3. Fernandes KG, da Silva BB, Boer NC, Mandarinini DR, Moreti LC, Kato AS, et al. The effectiveness of three irrigation systems in the *Enterococcus faecalis* reduction after instrumentation with a reciprocating instrument. Eur J Dent. 2020;14(4):539-43. doi: [10.1055/s-0040-1714760](https://doi.org/10.1055/s-0040-1714760).
  4. Alghamdi F, Shakir M. The influence of *Enterococcus faecalis* as a dental root canal pathogen on endodontic treatment: a systematic review. Cureus. 2020;12(3):e7257. doi: [10.7759/cureus.7257](https://doi.org/10.7759/cureus.7257).
  5. Gomes B, Aveiro E, Kishen A. Irrigants and irrigation activation systems in endodontics. Braz Dent J. 2023;34(4):1-33. doi: [10.1590/0103-6440202305577](https://doi.org/10.1590/0103-6440202305577).
  6. Tabassum S, Khan FR. Failure of endodontic treatment: the usual suspects. Eur J Dent. 2016;10(1):144-7. doi: [10.4103/1305-7456.175682](https://doi.org/10.4103/1305-7456.175682).
  7. Ashofteh K, Sohrabi K, Iranparvar K, Chiniforush N. In vitro comparison of the antibacterial effect of three intracanal irrigants and diode laser on root canals infected with *Enterococcus faecalis*. Iran J Microbiol. 2014;6(1):26-30.
  8. Mehta D, Choksi P, Vaidya R, Mistry K, Sanghvi Z, Patel P. Comparative evaluation of antibacterial efficacy of 5% sodium hypochlorite, 940 nm diode laser, and gaseous ozone against *Enterococcus faecalis* biofilm formed on tooth substrate: a scanning electron microscope study. J Oper Dent Endod. 2006;3(1):1-6. doi: [10.5005/jp-journals-10047-0047](https://doi.org/10.5005/jp-journals-10047-0047).
  9. Beer F, Buchmair A, Wernisch J, Georgopoulos A, Moritz A. Comparison of two diode lasers on bactericidity in root canals--an in vitro study. Lasers Med Sci. 2012;27(2):361-4. doi: [10.1007/s10103-011-0884-3](https://doi.org/10.1007/s10103-011-0884-3).
  10. Sohrabi K, Sooratgar A, Zolfagharnasab K, Kharazifard MJ, Afkhami F. Antibacterial activity of diode laser and sodium hypochlorite in *Enterococcus faecalis*-contaminated root canals. Iran Endod J. 2016;11(1):8-12. doi: [10.7508/iej.2016.01.002](https://doi.org/10.7508/iej.2016.01.002).
  11. Hendi SS, Shiri M, Poormoradi B, Alikhani MY, Afshar S, Farmani A. Antibacterial effects of a 940 nm diode laser with/without silver nanoparticles against *Enterococcus faecalis*. J Lasers Med Sci. 2021;12:e73. doi: [10.34172/jlms.2021.73](https://doi.org/10.34172/jlms.2021.73).
  12. Fahim SZ, Ghali RM, Hashem AA, Farid MM. The efficacy of 2780 nm Er,Cr:YSGG and 940 nm diode laser in root canal disinfection: a randomized clinical trial. Clin Oral Investig. 2024;28(3):175. doi: [10.1007/s00784-024-05563-z](https://doi.org/10.1007/s00784-024-05563-z).
  13. Asnaashari M, Tahmasebi Ebad L, Shojaeian S. Comparison of antibacterial effects of 810 and 980-nanometer diode lasers on *Enterococcus faecalis* in the root canal system--an in vitro study. Laser Ther. 2016;25(3):209-14. doi: [10.5978/islm.16-OR-17](https://doi.org/10.5978/islm.16-OR-17).
  14. Nanda Z, Singh R, Kamble PP, Deshmukh G, Patil N, Patil AB, et al. Efficacy of different root canal irrigating solutions in removing smear layer: a scanning electron microscopic study. Cureus. 2023;15(9):e44618. doi: [10.7759/cureus.44618](https://doi.org/10.7759/cureus.44618).
  15. Mohammadi Z, Shalavi S, Yaripour S, Kinoshita JI, Manabe A, Kobayashi M, et al. Smear layer removing ability of root canal irrigation solutions: a review. J Contemp Dent Pract. 2019;20(3):395-402.
  16. Sonisha S, Gaffoor FM, Gopakumar R, Girish CS, Mohan R, Anoop VN. Comparative evaluation of residual antibacterial substantivity of chlorhexidine, MTAD and chitosan against *Enterococcus faecalis* in human root dentin - an in vitro study. J Pharm Bioallied Sci. 2024;16(Suppl 2):S1400-3. doi: [10.4103/jpbs.jpbs\\_693\\_23](https://doi.org/10.4103/jpbs.jpbs_693_23).
  17. Torabinejad M, Khademi AA, Babagoli J, Cho Y, Johnson WB, Bozhilov K, et al. A new solution for the removal of the smear layer. J Endod. 2003;29(3):170-5. doi: [10.1097/00004770-200303000-00002](https://doi.org/10.1097/00004770-200303000-00002).
  18. Afkhami F, Akbari S, Chiniforush N. *Enterococcus faecalis* elimination in root canals using silver nanoparticles, photodynamic therapy, diode laser, or laser-activated nanoparticles: an in vitro study. J Endod. 2017;43(2):279-82. doi: [10.1016/j.joen.2016.08.029](https://doi.org/10.1016/j.joen.2016.08.029).
  19. Sher BM, Mulder R, Gutknecht N. The photobiomodulation effect of 940nm laser irradiation on *Enterococcus faecalis* in human root dentin slices of varying thicknesses. J Lasers Med Sci. 2021;12:e48. doi: [10.34172/jlms.2021.48](https://doi.org/10.34172/jlms.2021.48).
  20. Buraihi MM, Alkurtas SA. The photothermal effect of 940nm diode laser on *Enterococcus faecalis* biofilm in infected root canal. J Res Med Dent Sci. 2020;8(7):480-6.
  21. Schulte-Lünzum R, Gutknecht N, Conrads G, Franzen R. The impact of a 940nm diode laser with radial firing tip and bare end fiber tip on *Enterococcus faecalis* in the root canal wall dentin of bovine teeth: an in vitro study. Photomed Laser Surg. 2017;35(7):357-63. doi: [10.1089/pho.2016.4249](https://doi.org/10.1089/pho.2016.4249).
  22. Castelo-Baz P, Martín-Biedma B, Ruíz-Piñón M, Rivas-Mundiña B, Bahillo J, Seoane-Prado R, et al. Combined sodium hypochlorite and 940 nm diode laser treatment against mature *E. faecalis* biofilms in-vitro. J Lasers Med Sci. 2012;3(3):116-21.
  23. Shirani Lapari S, Zare Jahromi M, Tahmourespour A, Shirani AM. Comparison of the *Enterococcus faecalis* colony reduction effect of two wavelengths of diode lasers with three methods of root canal irrigation: an in vitro study. J Lasers Med Sci. 2024;15:e37. doi: [10.34172/jlms.2024.37](https://doi.org/10.34172/jlms.2024.37).
  24. Sarda RA, Shetty RM, Tamrakar A, Shetty SY. Antimicrobial efficacy of photodynamic therapy, diode laser, and sodium hypochlorite and their combinations on endodontic pathogens. Photodiagnosis Photodyn Ther. 2019;28:265-72. doi: [10.1016/j.pdpdt.2019.09.009](https://doi.org/10.1016/j.pdpdt.2019.09.009).
  25. Dubey S. Comparative antimicrobial efficacy of herbal alternatives (*Emblica officinalis*, *Psidium guajava*), MTAD, and 2.5% sodium hypochlorite against *Enterococcus faecalis*: an in vitro study. J Oral Biol Craniofac Res. 2016;6(1):45-8. doi: [10.1016/j.jobcr.2015.12.010](https://doi.org/10.1016/j.jobcr.2015.12.010).
  26. Srikumar GP, Sekhar KS, Nischith KG. Mixture tetracycline citric acid and detergent - a root canal irrigant. A review. J Oral Biol Craniofac Res. 2013;3(1):31-5. doi: [10.1016/j.jobcr.2012.09.001](https://doi.org/10.1016/j.jobcr.2012.09.001).
  27. Nara A, Dhanu, Chandra P, Anandakrishna L, Dhananjaya. Comparative evaluation of antimicrobial efficacy of MTAD, 3% NaOCl and propolis against *E. faecalis*. Int J Clin Pediatr Dent. 2010;3(1):21-5. doi: [10.5005/jp-journals-10005-1049](https://doi.org/10.5005/jp-journals-10005-1049).
  28. Swapna Priya N, Silpa Cherukulath H, Keerthinmayee K, Sonia Bai JK, Srikanth Reddy C, Lakshmi Sravya SN. Comparison of antibacterial efficacy of various root canal irrigants against *Enterococcus faecalis*: an invitro study. Int J Acad Med Pharm. 2024;6(1):420-5. doi: [10.47009/jamp.2024.6.1.81](https://doi.org/10.47009/jamp.2024.6.1.81).