

## ORIGINAL RESEARCH



## Antimicrobial Potential of Laser Diode in Infected Dentin

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### ABSTRACT

**Aim:** To evaluate the antibacterial effect of diode laser, associated or not with 2.5% sodium hypochlorite (NaOCl), against *Enterococcus faecalis*.

**Materials and methods:** Eighty dentin blocks were obtained from single-rooted human teeth and sterilized. Seventy were inoculated with 0.01 mL of fresh bacterial inoculum (within 24 hours of preparation from pure culture) standardized to 1 McFarland turbidity. Contaminated blocks were incubated for 7 days at 37°C in humid conditions. Ten uncontaminated samples were incubated at 37°C during the contamination period to serve as a negative control group, while 10 of the infected specimens served as a positive control group. The dentin blocks were randomly divided into eight experimental groups (n = 10 each) according to the method of decontamination: 2.5% NaOCl alone; 2.5% NaOCl + photodynamic therapy (PDT) with methylene blue/660 nm laser at 18 J for 180 seconds; 2.5% NaOCl + PDT with methylene blue/660 nm laser at 8 J for 80 seconds; methylene blue alone; PDT alone with methylene blue/660 nm laser at 18 J for 180 seconds; PDT alone with methylene blue/660 nm laser at 8 J for 80 seconds; positive control group; and negative control group. Microbial growth was evaluated by culture medium turbidity and microbial concentration was analyzed by UV spectrophotometry (adjusted to read at wavelength  $\lambda = 600$  nm).

**Results:** Root canals treated with laser alone at 18 J for 180 seconds had higher bacterial contamination compared with groups in which NaOCl was used, with or without laser irradiation at 18 J for 180 seconds ( $p < 0.05$ ).

**Conclusion:** Photodynamic therapy with a 660 nm diode laser effectively reduced *E. faecalis* contamination. These findings

can guide development of further studies in search of better alternatives for endodontic treatment.

**Clinical relevance:** Chemical and mechanical root canal preparation plays an essential role in reducing microbial burden. However, microorganisms present in areas not mechanically reachable by endodontic instruments. As an alternative to fix this problem, the laser can be applied.

**Keywords:** Dentin, *Enterococcus faecalis*, Phototherapy.

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### INTRODUCTION

Chemical and mechanical root canal preparation play an essential role in reducing microbial burden. However, microorganisms present in areas not mechanically reachable by endodontic instruments, such as the dentinal tubules, isthmus, lateral canals, and apical ramifications, can lead to treatment failure.<sup>1</sup> *Enterococcus faecalis* is a facultative anaerobic Gram-positive bacterium rarely found in cases of primary endodontic infections, but which may represent 38 to 70% of the microbiota in cases of retreatment.<sup>2</sup> *Enterococcus faecalis* has a unique biofilm formation strategy, virulence factors, adhesion to dentin collagen, survival in critical medium, and resistance to endodontic therapy.<sup>3</sup>

In endodontics, microbial elimination is a particular challenge due to the complexity of the root canal system.<sup>4,5</sup> Periapical lesions that persist after appropriate endodontic treatment are caused by the permanence of pathogenic bacteria within the root canals.<sup>6</sup> Irrigation is a critical part of the canal cleaning process, and irrigant solutions are expected to act in areas where endodontic instruments cannot reach.<sup>4,7</sup> A variety of solutions have

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been proposed, but none possesses all desired characteristics of an optimal irrigant; thus, various combinations of products and protocols have been tested.<sup>8</sup>

Photodynamic therapy stands out as a potential therapeutic approach to improve root canal cleaning.<sup>9</sup> This therapy is based on the organic reaction of photooxidation.<sup>9</sup> Application of a photosensitizing agent is followed by a dose of low-level laser radiation at a suitable wavelength.<sup>10</sup> The absorption of light activates the photosensitizer, which, in the presence of oxygen, triggers a cascade of photochemical effects that result in the production of highly reactive oxygen species, which are toxic to tumor cells, bacteria, and fungi.<sup>10</sup>

Within this context, the purpose of present study was to evaluate the antibacterial effect of diode laser irradiation, combined or not with 2.5% sodium hypochlorite (NaOCl) irrigation, against *E. faecalis* in contaminated human dentin blocks.

## MATERIALS AND METHODS

This study was approved by Research Ethics Committee of Universidade Federal de Goiás, Brazil (CAAE 19811113.0.0000.5083).

### Dentin Blocks Preparation

Selected teeth had fully formed roots and exhibited no defects, no internal or external root resorption, and no calcified canals. These criteria were checked through radiographic examination, mesiodistal and buccolingual viewing through a magnifying glass, and exploration of the root canal with a #15 K-file. Sequentially, eighty dentin blocks were obtained from single-rooted human teeth extracted for periodontal or prosthetic reasons.

The extracted teeth were placed in a 0.2% thymol solution until the dentin blocks could be obtained. Roots were sectioned on an Extec Labcut 1010 Low Speed cutting machine with a diamond saw (Buehler, Illinois, USA) under abundant air/water cooling. Dentin disks obtained from the cervical third of the root were cut into four specimens to yield dentin blocks 4 mm in height × 4 mm in length. The dimensions were verified using a digital caliper (Mitutoyo, São Paulo, Brazil) and, if necessary, corrected with a fine polishing disk (3M ESPE Dental Products, Minnesota, USA). Once each block was properly dimensioned, its outer surface was insulated with two layers of nail polish (Max Factor Cosmetics and Fragrances, London, UK) to prevent infiltration. The specimens were washed twice by sonication (Cristofoli, Paraná, Brazil) for 5 minutes in 17% ethylenediaminetetraacetic acid (Fórmula and Ação, São Paulo, Brazil) and left to soak for 10 minutes in sterile distilled water. The dentin blocks were sterilized in an autoclave (Cristofoli, Paraná,

Brazil) for 30 minutes at 120°C and incubated in 7 mL of Brain Heart Infusion (BHI) medium (Difco Laboratories, Michigan, USA) at 37°C for 48 hours to ensure sterilization. After this period, no bacterial growth was observed.

### Biological Indicator

For the present study, samples of the microorganism *E. faecalis* (ATCC 29212) were selected. The strain was inoculated into 7 mL BHI and incubated at 37°C for 24 hours. The microorganism was also grown in BHI surface under the same incubation conditions. Microbial cells were suspended in saline to McFarland turbidity standard 1, giving a final concentration of approximately  $3 \times 10^8$  cells/mL.

### Contamination of Dentin Blocks

The dentin blocks were inoculated with 0.01 mL of the aforementioned bacterial inoculum at 24 hours of preparation. Contaminated blocks were incubated for 7 days, at 37°C, in humid conditions.

Ten uncontaminated samples were incubated at 37°C during the contamination period to test the sterility of the samples that would serve as the negative control group. Ten infected specimens were likewise incubated at 37°C throughout the trial period to serve as a positive control group and assess the viability of the biological indicator.

### Experimental Irrigant

The irrigant solution tested in this experiment was 2.5% NaOCl (Plant Protection, GO, Brazil).

### Photodynamic Therapy

For the purposes of this study, PDT was administered by application of a low-level diode laser in the red spectrum (wavelength 660 nm; SiroLaser, Dentsply Sirona, Bensheim, Germany). Irradiation was performed in continuous mode at two power levels and durations: 18 J (for 180 seconds) and 8 J (for 80 seconds).

### Study Design

Contaminated dentin blocks were randomly divided into six groups, each of which received one of the following decontamination methods:

Group I (NaOCl alone): Dentin blocks were submerged in 5 mL of 2.5% NaOCl solution for 5 minutes. Subsequently, each block was rinsed with 5 mL of sterile distilled water, transferred individually into 7 mL BHI to which sodium thiosulfate (PA Art Laboratories, São Paulo, Brazil) and Tween 80 were added in appropriate concentrations, and incubated at 37°C for 48 hours.

Groups II and III (NaOCl plus PDT): Dentin blocks were submerged in 5 mL of 2.5% NaOCl solution for

5 minutes. Subsequently, each block was rinsed with 5 mL of sterile distilled water, transferred individually into Dappen dishes containing 0.001% methylene blue as photosensitizer (Phloraceae Farmácia de Manipulação, Cuiabá, Mato Grosso, Brazil), where they remained for 3 minutes (preirradiation period). Then, specimens were again rinsed with 5 mL of sterile distilled water, and laser irradiation was performed. After irradiation, each block was transferred individually into 7 mL BHI to which sodium thiosulfate (PA Art Laboratories, São Paulo, Brazil) and Tween 80 were added at appropriate concentrations, and incubated at 37°C for 48 hours. Group II specimens received diode laser irradiation at 18 J for 180 seconds, while group III specimens were irradiated at 8 J for 80 seconds.

Group IV (Methylene blue alone): Dentin blocks were submerged in 0.001% methylene blue, where they remained for 3 minutes, followed by rinsing with 5 mL of sterile distilled water. The specimens were then transferred individually into 7 mL BHI to which sodium thiosulfate and Tween 80 were added at appropriate concentrations, followed by incubation at 37°C for 48 hours.

Groups V and VI (PDT alone): Dentin blocks were submerged in 0.001% methylene blue, where they remained for 3 minutes, followed by rinsing with 5 mL of sterile distilled water and laser irradiation. After irradiation, each block was transferred individually into 7 mL BHI to which sodium thiosulfate and Tween 80 were added at appropriate concentrations, and incubated at 37°C for 48 hours. Group V specimens received diode laser irradiation at 18 J for 180 seconds, while group VI specimens were irradiated at 8 J for 80 seconds.

### Microbiological Analysis

Microbial growth was assessed by turbidity of the culture medium. Thereafter, a 0.1 mL inoculum of the BHI obtained was transferred to 5 mL of Letheen Broth under identical incubation conditions. Gram staining was performed on all BHI cultures to check for contamination and growth, and all were examined macroscopically and microscopically. The microbial concentration was analyzed in a UV spectrophotometer (New Model 1600 UV, São Paulo, Brazil) set to read at a wavelength of 600 nm.

### Statistical Analysis

All results were expressed as means and analyzed via the nonparametric Kruskal–Wallis and Mann–Whitney tests (Wilcoxon rank-sum tests) at the 5% significance level.

## RESULTS

Table 1 lists means and standard deviations (SDs) for the spectrophotometry data assessed in the different

**Table 1:** Means and SDs of spectrophotometry values for specimens in each disinfection protocol group, before (initial) and after (final) disinfection

Disinfection protocol	Initial	Final
2.5% NaOCl	0.818 ± 0.086 <sup>a</sup>	0.181 ± 0.098 <sup>b</sup>
2.5% NaOCl + PDT with methylene blue/18 J laser (180 sec)	0.781 ± 0.054 <sup>a</sup>	0.208 ± 0.011 <sup>b</sup>
2.5% NaOCl + PDT with methylene blue/8 J laser (80 sec)	0.863 ± 0.043 <sup>a</sup>	0.207 ± 0.127 <sup>b</sup>
Methylene blue	0.772 ± 0.031 <sup>a</sup>	0.364 ± 0.038 <sup>b</sup>
PDT with methylene blue/18 J laser (180 sec)	0.874 ± 0.041 <sup>a</sup>	0.342 ± 0.020 <sup>b</sup>
PDT with methylene blue/8 J laser (80 sec)	0.779 ± 0.048 <sup>a</sup>	0.356 ± 0.018 <sup>b</sup>

Different letters indicate statistically significant differences between columns

experimental conditions, for both initial (before root canal cleaning) and final analysis (after root canal cleaning). Analysis of the distribution of random errors around the mean by the Shapiro–Wilk test showed a non-normal distribution ( $p < 0.05$ ) at the initial and final time points. Thus, for comparison of data between different root canal cleaning methods at each time point (unpaired samples), the Kruskal–Wallis test was used; statistically significant differences ( $p < 0.001$ ) were found before and after sanitization. Given these results, the comparison of spectrophotometry data after root canal cleaning could be biased, as differences were already present at the initial time point. Thus, the least squares method was used for correction of final spectrophotometry data. To compare data between the initial and final periods (paired data), Wilcoxon’s rank-sum test was used, and revealed a statistically significant difference ( $p < 0.001$ ). After root canal cleaning, there was a significant reduction in microbial burden, as revealed by lower values found on spectrophotometry.

Initially, a linear regression analysis between the initial and final data was carried out to check for correlation between them. This analysis showed no statistical significance ( $p < 0.001$ ). The angular coefficient was recorded (0.434) and a standard initial spectrophotometry value was obtained by averaging the values of the different groups (0.817). These data were then entered into the following equation:  $[\text{Spectrophotometry}_{\text{adjusted}} = \text{final specimen spectrophotometry value} + \text{angular coefficient} * (\text{initial specimen spectrophotometry} - \text{standard spectrophotometry value})]$ .

Table 2 shows the means and SDs of the data obtained with this calculation. This new dataset, now adjusted to the standard initial spectrophotometry value (except for the negative control group), was analyzed as to the distribution of random errors around the mean by the Shapiro–Wilk test, which rejected the assumption of

**Table 2:** Means and SDs of adjusted spectrophotometry values for specimens in each disinfection protocol group

Disinfection protocol	Adjusted spectrophotometry value
2.5% NaOCl	0.181 ± 0.098 <sup>a,c</sup>
2.5% NaOCl + PDT with methylene blue/18 J laser (180 sec)	0.192 ± 0.127 <sup>a,c,d</sup>
2.5% NaOCl + PDT with methylene blue/8 J laser (80 sec)	0.226 ± 0.122 <sup>a,b,c,d</sup>
Methylene blue	0.344 ± 0.041 <sup>a,e</sup>
PDT with methylene blue/18 J laser (180 sec)	0.367 ± 0.032 <sup>b,e</sup>
PDT with methylene blue/8 J laser (80 sec)	0.339 ± 0.029 <sup>a,e</sup>
Positive control	0.860 ± 0.083 <sup>e</sup>
Negative control	0.000 ± 0.000 <sup>c</sup>

Different letters indicate statistically significant differences between the disinfection protocols

normality ( $p < 0.05$ ). Therefore, data were again analyzed using the Kruskal–Wallis test, which revealed statistically significant differences for different root canal cleaning methods ( $p < 0.001$ ). The nonparametric Tukey test revealed that bacterial contamination was higher in the positive control group than in all others ( $p < 0.05$ ), except those in which laser alone was used, regardless of the parameters employed, and the group in which methylene blue alone was used ( $p > 0.05$ ). Root canals irradiated with the laser settings 18 J/180 seconds had higher bacterial contamination than the negative control group and the groups in which NaOCl was employed, with or without laser at 18 J/180 seconds ( $p < 0.05$ ). Differences were also found between the laser 8 J/80 seconds and negative control group, as well as between methylene blue alone and negative control ( $p < 0.05$ ). The other methods did not differ ( $p < 0.05$ ).

## DISCUSSION

The results of this study showed a reduction in microbial burden after root canal cleaning, with significant differences ( $p < 0.001$ ) among the different tested methods. As expected, the Tukey test showed that the positive control group had the highest bacterial contamination ( $p < 0.05$ ). Root canals cleaned with a 660-nm diode laser at 18 J for 180 seconds had higher bacterial contamination than the negative control group and the groups in which NaOCl irrigation was employed, with or without laser irradiation ( $p < 0.05$ ). Differences were also found between laser irradiation at 8 J for 80 seconds and negative control, as well as between cleaning with methylene blue alone and negative control ( $p < 0.05$ ). *Enterococcus faecalis* was chosen as the biomarker of contamination based on its presence in the root canal system, especially in teeth with endodontic treatment failure.<sup>11-16</sup> The methodology applied in this

study has been previously employed elsewhere, and the use of *E. faecalis* as a 7-day-old biofilm has already been described.<sup>17,18</sup>

The optimal irrigant solution and its ideal concentration have yet to be defined. Although some studies have shown that the optimal concentration of NaOCl for use in endodontic treatment ranges from 1 to 2.5%, others suggest that chlorhexidine is the ideal irrigant;<sup>8,19</sup> additional studies are still needed to elucidate this controversy. Taking into account the need for root canal disinfection and the lack of consensus on the best irrigant solution, concentration, and application protocol, PDT has been considered a viable alternative for endodontic cleaning.<sup>20,21</sup> The PDT can be used as an adjunct to conventional endodontic treatment without damaging the cells of the periapical region.<sup>22</sup> Furthermore, compared with conventional antimicrobial therapy, it has the advantage of not triggering any mechanisms of microbial resistance, and can be used numerous times, as it shows no cumulative effect.<sup>21,22</sup> All macromolecules of microbial cells are potential targets for highly reactive oxygen species.<sup>23</sup> Thus, the antimicrobial effect of PDT has been the subject of several studies.<sup>8,15,24,25</sup>

The antimicrobial effects of different disinfection protocols with or without the use of PDT have been evaluated. Even in protocols that employed PDT alone, reductions in microbial burden were verified.<sup>26-28</sup> Other studies compared PDT with different irrigant solutions used during endodontic treatment, and found better results for microbial reduction.<sup>15</sup> Soukos et al<sup>26</sup> investigated the effect of PDT with a diode laser at a wavelength of 665 nm in the root canals of extracted and infected teeth. The results showed the inoculation of microorganisms, with the exception of *E. faecalis*, for which only a reduction was achieved. The difference is likely to be associated with the organization of *E. faecalis* in the form of a biofilm, as reported elsewhere.<sup>10</sup> Pinheiro et al<sup>29</sup> studied the antimicrobial action of diode-laser PDT in primary teeth with pulp necrosis after conventional endodontic treatment. Conventional endodontic treatment reduced the microbial burden by 82.59%; after PDT, a 98.37% reduction was achieved. Garcez et al<sup>30</sup> evaluated the effect of PDT in endodontic retreatment, and concluded that the combination of PDT with conventional endodontic treatment reduces microorganism counts. Queiroga et al<sup>31</sup> evaluated the effectiveness of different laser dosages (60, 120, and 180 J/cm<sup>2</sup>) on *Candida* spp., and concluded that the higher doses were most effective, achieving 78% reductions in microbial burden. Vaziri et al<sup>32</sup> analyzed the combined use of PDT and 2.5% NaOCl irrigation and found complete elimination of *E. faecalis* in the canal systems of single-rooted teeth. Filipov et al<sup>33</sup> investigated the antimicrobial effect of PDT on 5-day-old *E. faecalis* biofilm and *Candida*

*albicans*, by scanning electron microscopy, and concluded that it has potential to be a good alternative or adjunct to conventional root canal disinfection methods.

The PDT can be used as an adjunct to endodontic treatment,<sup>1</sup> but for maximum effectiveness to be achieved with this therapy, additional studies should be conducted to determine the optimal settings for parameters, such as dose, power, photosensitizer molecule and concentration, pre-irradiation time, and exposure.

## CONCLUSION

The PDT with a diode laser at the 660 nm wavelength effectively reduced *E. faecalis* burden. However, root canals treated solely with laser irradiation at 18 J for 180 seconds, without irrigation, had higher bacterial contamination than groups in which NaOCl irrigation was employed, with or without laser irradiation.

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