

# Effectiveness of high-power diode laser associated to chemical auxiliary substance on decontamination of root canal system infected with *Enterococcus faecalis* – an in vitro study

*Eficácia do laser diodo de alta potência associado com substâncias químicas auxiliares na descontaminação do sistema de canais radiculares infectados com *Enterococcus faecalis* – estudo in vitro*

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## Abstract

*Objective: to evaluate the additional antibacterial action of high-power diode laser after the use of 2.5% sodium hypochlorite and 2% chlorhexidine gel in root canals infected with E.faecalis. Materials and method: 120 human teeth with single canal were inoculated with E.faecalis for 14 days and randomly divided into six groups (n=20), according to the chemical auxiliary substance used in the chemo-mechanical preparation and the use of high-power diode laser: G1(NaOCl) – root canal preparation with 2.5% sodium hypochlorite; G2(CHX) – root canal preparation with 2% chlorhexidine gel; G3(NaOCl+DL) - root canal preparation with 2.5% sodium hypochlorite+diode laser; G4(CHX+DL) - root canal preparation with 2% chlorhexidine gel+diode laser; G5(NaCl+DL) - root canal preparation with 0.9% saline solution+diode laser; G6(no treatment) – no treatment was performed. Microbiological test (CFUs counting) was performed*

*to evaluate the effectiveness of proposed treatments. Data were subjected to One-way ANOVA followed by post-hoc Tukey test ( $\alpha=0.05$ ). Results: G3(NaOCl+DL) had reduction of 99.42% of E.faecalis, followed by G4(CHX+DL) – 93.48%; G1(NaOCl) – 89.82%; G2(CHX) – 81.86%; G5(NaCl+DL) – 65.40%; and G6(no treatment) – no reduction. There was significant statistical difference in CFUs of all groups in relation to G6(no treatment) ( $p<0.05$ ). G1(NaOCl), G2(CHX), G3(NaOCl+DL) and G4(CHX+DL) were statistically superior to G5(NaCl+DL) ( $p<0,05$ ). Conclusion: High-power diode laser, when associated with 2.5% NaOCl or 2% CHX, improves the antimicrobial effect of these substances in the root canals infected with E. faecalis.*

*Keywords: Laser. Sodium hypochlorite. Chlorhexidine, Enterococcus faecalis.*

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## Introduction

The vast majority of endodontic failures is associated with some shortcoming in the decontamination process and maintenance of microorganisms in the root canal system. Literature shows that success rates of endodontic retreatments are lower than conventional root canal therapy, around 75%<sup>1</sup>. This fact is related to some difficulties in the decontamination process, due to a distinct microbiota present in these cases.

The *Enterococcus faecalis* (*E. faecalis*) is an anaerobic facultative bacteria which grows in a biofilm structure on the root canal walls and on the depth of dentinal tubules, being capable to proliferate in either the presence or absence of oxygen<sup>2,3</sup>. This microorganism has an extra polysaccharide matrix which provides mechanical stability and decreases the action of adjuvant substances in root canal therapy<sup>5,6</sup>. Furthermore, *E. faecalis* can survive for 10 days within dentinal tubules with no nutrient source<sup>1</sup> and stay viable into filled root canals until 12 months after root canal therapy<sup>7</sup>.

Thus in addition to physic and chemical modes, a different approach is necessary in order to promote an adequate decontamination of root canal system. The laser therapy arises as an auxiliary for the root canal treatment<sup>8-11</sup>. Several studies have evaluated the effectiveness of high-power lasers, such as diode laser and ErCr:YSGG, in the decontamination process of radicular dentin, showing its possible applications in endodontics<sup>6,8,12</sup>. The energy of high-power laser can be transmitted through the root canal by an optic fiber which has the ability to reach inaccessible areas of the root canal system, where endodontic instruments and chemical auxiliary substance can not properly work. Likewise, diode laser is easy to transport and may be considered a more economical alternative when compared with other high-power lasers such as Er,Cr:YSGG, Nd:YAG and CO<sub>2</sub>, which justifies its testing as an antimicrobial alternative in the root canal therapy.

The purpose of this study was to assess the additional antibacterial action of high-power diode laser after the use of 2.5% sodium hypochlorite and 2,0% chlorhexidine gel in root canals infected with *E. faecalis*.

## Materials and method

This study was approved by Ethics Committee in Research of University of Passo Fundo (Passo Fundo, RS, Brazil) (protocol 229/2011).

## Sample obtainment and specimen preparation

One hundred and thirty extracted human lower premolars with a single root canal were used for the present study. The teeth were stored in 4% formalin in order to preserve their properties. The dental crowns were sectioned so that all the roots had 13 mm in length. All roots were prepared using the same protocol in order to remove the pulp tissue and to standardize the canal diameter. The cervical third were prepared using Gates-Glidden #3 drill (Dentsply-Maillefer, Ballaigues, Switzerland). The working length was established by introducing a K-file #10 in the canal until its tip was visualized at the apical foramen. The working length was established by reducing 1 mm from this measure. The roots were prepared up to the K-file #30 (Dentsply-Maillefer, Ballaigues, Switzerland), by serial instrumentation, using irrigation with 2.5% sodium hypochlorite (Lírio Química Ltda, São Vicente, SP, Brazil). Then, a final rinse with 17% EDTA (Iodontosul, Porto Alegre, RS, Brazil) was performed for smear layer removal. The roots were coated externally with cyanoacrylate (SuperBonder, Loctite Henkel, Itapevi, SP, Brazil), dried at room temperature for 24 hours and fixed in a plastic micro-tube of 1.5 mL. The samples were sterilized in autoclave (Dabi Atlante, Ribeirão Preto, SP, Brazil) for a period of 30 minutes at a temperature of 120 °C.

## Sterilization control

Ten samples were randomly chosen and filled with sterilized Ringer solution. Then, the absorbent paper points were removed from contact with root canal walls after 15 seconds and individually transported to a plastic micro-tube (GenuineAxygen-Quality, CA, USA) containing 1 mL of 0.9% saline solution (Basso, Caxias do Sul, RS, Brazil). The material was homogenized and after five minutes an aliquot of 100 µL of saline solution was cultivated on blood agar with the aid of a Drigalski handle, in duplicate. The samples were incubated at 37 °C for 48 hours, in order to verify the bacterial growth.

## Inoculum preparation and root canal contamination

In an essay tube containing 5 mL of Tryptic Soy Broth (TSB, Merck, Frankfurt, Germany) 10 µL of the culture of *E. faecalis* (ATCC29212) were inoculated. The tube was incubated at 37°C for 24 hours in an incubator. The turbidity level of inoculum was adjusted according to grade 1.0 into the McFarland scale<sup>6</sup>, using Tryptic Soy Broth.

In each one of the 120 samples previously sterilized, 0.8 mL of the inoculum containing *E. faeca-*

lis was inoculated inside the root canal until total filling, using disposable syringes (Med-Inject, São José, SC, Brazil). Then, a new culture with equal turbidity was injected into the root canal every 24 hours for a period of 14 days. After each renewal, the samples were stored at 37 °C in an incubator in order to promote biofilm formation.

## Contamination control

Twenty samples were randomly chosen for contamination control. Sterilized paper points #25 were inserted into the root canal of each sample for a period of 15 seconds. After that, the paper point was placed into the Eurotype tubes containing 1 ml of 0.9% saline solution, sonicated for 20 seconds. Serial dilutions at 10<sup>-4</sup> were performed. From the

diluted sample, an aliquot of 25µL was cultivated in plates containing Agar Plate Count Agar (AES, Combourg, France) with the aid of a Drigalski handle in duplicate. The plates were incubated at 37 °C for 24 hours in an incubator. Catalase and oxidase tests (Newprov, Pinhais, PR, Brazil), and Gram coloration were performed in order to evaluate the morpho-tinctorial characterization of colonies of *E. faecalis*.

## Classification of treatment groups

The 120 samples were randomly divided into six groups (n=20) according to the chemical auxiliary substance used in the chemo-mechanical preparation, as well as the use of high-power diode laser, as showed in Table 1.

Table 1 - Groups distribution according to the chemical auxiliary substance used in the chemo-mechanical preparation and to the use or not of the high-power diode laser

Group	N	Chemo-mechanical preparation	Chemical auxiliary substance	Laser diode
G1 (NaOCl)	20	Yes	2.5% sodium hypochlorite*	No
G2 (CHX)	20	Yes	2.0% chlorhexidine gel**	No
G3 (NaOCl+DL)	20	Yes	2.5% sodium hypochlorite*	Yes
G4 (CHX+DL)	20	Yes	2.0% chlorhexidine gel**	Yes
G5 (NaCl+DL)	20	Yes	0.9% saline solution***	Yes
G6 (no treatment)	20	No	None	No
Total	120			

\* (Lírio Química Ltda, São Vicente, SP, Brazil)

\*\* (Essencial Pharma, Itapetininga, SP, Brazil)

\*\*\* (Basso, Caxias do Sul, RS, Brazil)

## Root canal preparation and laser application

The working length was established by introducing a K-file #10 into the root canal until its tip was visualized at the apical foramen. The working length was established by reducing 1 mm from this measure. The root canals were prepared by the crown-down technique, adopting the following sequence of instruments: Sx, F2 and F3 files of ProTaper rotary system (Dentsply-Maillefer, Ballaigues, Switzerland), followed by K-files #35 and #40. The rotary files were activated by X-Smart Endodontic Motor (Dentsply-Maillefer, Ballaigues, Switzerland), with 300 RPM of speed and 2 N/cm<sup>2</sup> of torque.

In the G1(NaOCl) and G3(NaOCl+DL), 2.5% sodium hypochlorite was used as chemical auxiliary substance in the root canal preparation. The root canal was filled with this solution and irrigation with 4 ml of the same substance was performed after the action of each instrument. In the G2(CHX) and G4(CHX+DL), 2,0% chlorhexidine gel (Essencial Pharma, Itapetininga, SP, Brazil) was used as auxiliary chemical substance. The root canal was filled with this substance and irrigation with 4 ml of

0.9% saline solution was performed after the action of each instrument. In the G5(NaCl+DL), 0.9% saline solution was used as chemical auxiliary substance in the root canal preparation. The root canal was filled with this solution and irrigation with 4 mL of the same substance was performed after the action of each instrument. After the action of the last file, irrigation with 5 ml of 17% EDTA (Iodontosul, Porto Alegre, RS, Brazil) was performed, followed by irrigation with 5 mL of 0.9% saline solution. Then, the root canals were dried with sterilized absorbent paper points.

After the chemo-mechanical preparation, the samples of G3(NaOCl+DL) and G4(CHX+DL), were irradiated with a diode laser (Opus 10, OpusDent, Sharplan Laser Industries, Ltd., Tel-Aviv, Israel), with a total power of 3W and 830 nm of wavelength. The helical movement technique developed by Gutknecht *et al.*<sup>13</sup> (1996) was performed in these groups. The optic fiber was placed 1 mm short of working length and removed with 2 mm/s of speed, in helical movements, in an apical-coronal direction, for 6 seconds in each application. Four cycles of irradiation were performed with an interval of 10 seconds between each application.

## Microbiological analysis

After laser application, the root canal was immediately filled with sterilized Ringer solution. Sterilized absorbent paper points were inserted into the root canal at working length. Then, the absorbent paper points were removed from contact with the root canal walls after 15 seconds and individually transported to a plastic micro-tube (GenuineAxygenQuality, CA, USA) containing 1 mL of 0.9% saline solution. The content was homogenized and diluted to  $10^{-3}$ . Aliquots of 100  $\mu$ L of the dilution were cultivated on the surface of the blood agar, in duplicate, with the aid of a Drigalski handle, being incubated for 18 to 24 hours at 37 °C. After the incubation period, the counting of the number of colony-forming units of the plates was performed.

## Data analysis

In the microbiology analysis, all data were log transformed. One-way ANOVA was applied on these data followed by Tukey's post hoc procedure. The level of significance was set at  $\alpha=0.05$ . Data were analyzed using SPSS version 17.0.

## Results

The results of microbiological analysis are expressed in the Table 2. G6 (no treatment) showed an average count of 2.43  $\log_{10}$  CFU/mL, being statistically different from all other groups ( $p<0.05$ ). There was no statistically significant difference between G1(NaOCl), G2(CHX), G3(NaOCl+DL) and G4(CHX+DL). However, these groups were statistically different when compared to G5(NaCl+DL), showing lower values of counting of the number of colony-forming units ( $p<0.05$ ).

Table 2 - Mean and standard deviation of CFU/mL ( $\log_{10}$ ) of each group, and microbial reduction (%) of each group compared to G6 (no treatment).

Group	CFU/mL ( $\log_{10}$ )	Microbial reduction (%)
G1 (NaOCl)	1.25 <sup>A</sup> $\pm$ 0,45	89.82
G2 (CHX)	1.36 <sup>A</sup> $\pm$ 0,53	81.86
G3 (NaOCl+DL)	1.04 <sup>A</sup> $\pm$ 0,13	99.42
G4 (CHX+DL)	1.18 <sup>A</sup> $\pm$ 0,37	93.48
G5 (NaCl+DL)	1.78 <sup>B</sup> $\pm$ 0,48	65.40
G6 (no treatment)	2.43 <sup>C</sup> $\pm$ 0,11	-

Different letters indicate a statistically significant difference at the 5% level.

## Discussion

One of the most important factors for achieving endodontic success is an effective decontamination of root canal system. Chemical substances and alternative resources are available to perform this role concurrently with mechanical action of endodontic instruments.

The model of biofilm formation used in this study simulates the clinical conditions found in infected root canals. *Enterococcus faecalis* is usually found in persistent lesions even after root canal treatment<sup>14-16</sup>. Furthermore, this microorganism shows the ability to successfully colonize the root canal system in the biofilm form<sup>3,17</sup>, which justifies its selection for the tested specie of the present study.

Nowadays, endodontic studies are searching for effective ways to neutralize microorganisms such as *E. faecalis* from the root canal system. On this way, several laser systems have been tested as adjuncts to currently used disinfection methods in root canal treatment.

In the present study, a high-power diode laser was tested in order to evaluate the additional anti-

microbial effects of this alternative resource on root canal system infected with *E. faecalis*. The high-power lasers have shown to decrease the endodontic microbiota<sup>12,18,19</sup>. In addition, the diode laser is easy to transport and can be considered a more economical alternative when compared to other high-power lasers such as Er,Cr:YSGG, Nd:YAG and CO<sub>2</sub>. For these reasons, the diode laser was tested as an antimicrobial alternative in the present study, to promote the decontamination of root canal system.

Groups G3(NaOCl+DL) and G4(CHX+DL) showed lower counting of the number of colony-forming units than all other groups, although with no statistically significant difference when compared to G1(NaOCl) and G2(CHX) where the root canals were not irradiated. Similar results were found in previous studies<sup>6,20-22</sup> which referred that diode laser helped to reduce the number of bacteria in deep layers of infected root canal system, when associated to conventional chemo-mechanical preparation. This can be explained by the broad antimicrobial spectrum of sodium hypochlorite and chlorhexidine, demonstrated by previous studies<sup>23,24</sup>, as well as to the protein denaturation and photothermal action provided by high-power diode laser over the bacterial cell<sup>25</sup>.

High-power lasers have been tested as adjuncts to currently used disinfection methods in root canal treatment. However, these lasers may induce thermal injury in tissues, such as dentin carbonization, root ankylosis, cementum melting, root resorption and periradicular necrosis<sup>26-29</sup>. In the present study the irradiation with diode laser was performed through short cycles with an interval of 10 seconds between each application. Thus, there is no increase of temperature on the root canal surface according to previous study<sup>13</sup>.

The results of present study showed that the groups where 2.5% sodium hypochlorite was used as chemical auxiliary substance demonstrated a lower mean in the counting of CFUs when compared to 2.0% chlorhexidine gel, regardless of irradiation with diode laser. It can be explained by the lower superficial tension and liquid formulation of sodium hypochlorite, which provides a higher diffusion into root canal system and dentinal tubules.

Sodium hypochlorite is the most common chemical auxiliary substance in the root canal therapy. This substance has effective antimicrobial activity<sup>23</sup> and ability to dissolve organic tissues<sup>30</sup>. However, is extremely cytotoxic when extravasated to the periapical tissues<sup>31</sup>, promotes dentin deproteinization<sup>32</sup> and interferes negatively on bond strength between restorative material and dentin<sup>33</sup>. Chlorhexidine has been used as an alternative to chemical auxiliary substance in the root canal preparation its broad antimicrobial spectrum<sup>26</sup> and substantivity<sup>34</sup>. However, this substance is not able to dissolve pulp tissue<sup>35</sup>. According to present study, both chemical auxiliary substances were not able to reduce completely the microorganisms from the root canal system, when used without the aid of diode laser. These findings testify that alternative resources such as high-power diode laser are necessary to improve the decontamination of the root canal system, reaching inaccessible areas by conventional methods of chemo-mechanical preparation.

Despite the effectiveness of tested protocols, none of methods was able to reduce completely the microbial content of root canal system. G3(NaOCl+DL) and G4(CHX+DL) reached 99.42% and 93.48% of microbial reduction respectively, being considered the most effective protocols on the decontamination process. However, these protocols were not able to control the infection in an absolute way, even with mechanical action of endodontic instruments, chemical action of chemical auxiliary substance and the association of high-power diode laser. It can be explained by the high resistance of *E.faecalis* and its organization in a biofilm structure<sup>36</sup>, which can prevent its complete eradication from the root canal system.

The present study suggests the use of high-power diode laser as an adjunct to chemo-mechanical preparation with 2.5% sodium hypochlorite or 2.0%

chlorhexidine gel. In addition to the chemical action of the auxiliary chemical substance and mechanical action of the endodontic instruments, the high-power diode laser would be an additional resource with ability to reach complexity areas of the root canal system. Therefore, the removal of resistant microorganisms which are commonly found in cases of endodontic infection would be facilitated into the root canal system.

## Conclusion

Under the limitation of this study, it can be concluded that the use of high-power diode laser, when associated to 2.5% sodium hypochlorite or 2.0% chlorhexidine gel, improves the antimicrobial effect on root canals infected *E.faecalis*.

## Resumo

*Objetivo: avaliar a ação antibacteriana adicional do laser diodo de alta potência após o uso de hipoclorito de sódio 2,5% e clorexidina gel 2,0% em canais radiculares infectados com Enterococcus faecalis. Materiais e método: cento e vinte dentes humanos unirradiculares foram inoculados com Enterococcus faecalis por 14 dias e divididos aleatoriamente em 6 grupos (n=20), de acordo com a substância química auxiliar utilizada no preparo químico-mecânico e o uso do laser diodo de alta potência: G1(NaOCl) – PQM com hipoclorito de sódio 2,5%; G2(CHX) – PQM com clorexidina gel 2,0%; G3 (NaOCl+DL) – PQM com hipoclorito de sódio 2,5% + aplicação de laser diodo; G4 (CHX+DL) – PQM com clorexidina gel 2,0% + aplicação de laser diodo; G5 (NaCl+DL) – PQM com soro fisiológico 0,9% + aplicação de laser diodo; G6 (sem tratamento) – nenhum procedimento foi realizado. Teste microbiológico (contagem de UFCs) foi realizado para avaliar a eficácia dos tratamentos propostos. Os dados foram submetidos à análise estatística através de One-way ANOVA seguido por post-hoc de Tukey ( $\alpha=0,05$ ). Resultados: G3(NaOCl+DL) teve redução de 99,42% de Enterococcus faecalis, seguido por G4(CHX+DL) – 93,48%; G1(NaOCl) – 89,82%; G2(CHX) – 81,86%; G5(NaCl+DL) – 65,40%; and G6(sem tratamento) – sem redução. Houve diferença estatisticamente significativa na contagem de UFCs de todos os grupos em relação ao grupo 6 (sem tratamento) ( $p<0,05$ ). G1(NaOCl), G2(CHX), G3(NaOCl+DL) e G4(CHX+DL) foram superiores ao grupo G5 (NaCl+DL) ( $p<0,05$ ). Conclusão: Laser diodo de alta potência, quando associado ao NaOCl 2,5% ou clorexidina gel 2,0% aumenta o efeito antimicrobiano destas substâncias nos canais radiculares infectados com E. Faecalis.*

*Palavras-chave: Laser. Hipoclorito de sódio. Clorexidina. Enterococcus faecalis.*

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