



Adjuvant therapy with a 980-nm diode laser in root canal retreatment: randomized clinical trial with 1-year follow-up

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Abstract

The purpose of this study is to assess the laser effect in root canal disinfection and periapical healing of endodontically treated teeth from patients with asymptomatic apical periodontitis. This study was performed as a randomized clinical trial. Thirty patients were selected according to the inclusion/exclusion criteria. Fifteen patients received the root canal retreatment (RCR) combined with 980-nm diode laser irradiation (LI). The canals were irrigated with saline solution and gently dried with paper points, keeping the dentin partially moist. The irradiation was performed using a 320- μ m-diameter fiber in helicoidal movements (pulsed mode, power output of 1.5 W, 100 Hz for 20 s). The other 15 patients received the RCR with placebo irradiation (PI). Microbiological samples were taken in three periods: S1, after the filling material removal (baseline); S2, after laser or placebo irradiation (LI or PI); and S3, after the RCR followed by laser or placebo. The samples were submitted to the total microbial and *E. faecalis* counting. The periapical radiographic healing was analyzed after 3, 6, 9, and 12 months. Microbiological data (CFU/mg) were analyzed by ANOVA and Tukey's test ($P < 0.05$), and the repair by Mann–Whitney test ($P < 0.05$). In S2, the laser provided 42.44% microbial reduction and 53.14% of *E. faecalis*, different from the placebo that had no reduction, and 4.85% for *Enterococcus* ($P < 0.05$). In S3, the bacterial counts decreased without differences between groups. No differences in healing were found at 3 months. However, diode laser facilitated the repair from 3- to 12-month follow-up ($P < 0.05$) and had 45% more healed cases than placebo. Diode laser provided an antimicrobial effect before the biomechanical preparation but was not synergistic in RCR. It improved the periapical healing during follow-up.

Keywords Diode lasers · Disinfection · Endodontics · Periapical periodontitis

Introduction

Apical periodontitis is an inflammatory disease of microbial etiology, which results from a complex interplay between the host immune response and the presence of bacteria and their products in the periapical tissues [1, 2]. Anatomically challenging areas of the root canal system and bacterial bio-film development are of utmost importance in influencing

treatment outcomes and may cause persistent periapical lesions [3–5].

Different types of lasers can improve root canal disinfection [6, 7] because of the bactericidal effect, the ability to penetrate through the dentinal thickness, and the ability to vaporize soft tissues [6, 8]. Stimulation of bone formation in the periapical area has also been tested with promising results [9]. The effect of laser irradiation on biological tissues is determined by several parameters such as wavelength, pulse mode, optical fiber diameter, exposure time, energy radiation, and physical characteristics of the tissues [10].

A diode laser carries light through a thin and flexible fiber compatible with the shape and dimensions of the root canal [11, 12]. Laboratory studies have shown an antibacterial effect of 810- to 980-nm diode lasers on standard bacterial strains associated with endodontic infection [7, 8, 13–15]. Furthermore, this wavelength can penetrate inside the dentinal tubules where irrigating solutions cannot

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penetrate [7, 8, 13, 16], reduce the postoperative pain [17, 18], remove the smear layer [10, 11], and consequently improve sealer penetration into dentinal tubules [11, 14, 19].

Clinical results have shown bacterial reduction with diode laser in the wavelength range of 635–808 nm after photodynamic therapy (PDT) [3, 6, 20, 21]. Unfortunately, the low-power and low-wavelength irradiation protocols depend on applying a membrane-targeting dye photosensitizer that identifies target cells to the light source [4].

To the best of our knowledge, only three clinical studies [22–24] have evaluated the antimicrobial effect of the high-power 980-nm diode laser in root canals. However, none of these studies assessed the influence of this laser in persistent infections (retreatment cases) or long-term bone repair. This clinical trial evaluated the effect of 980-nm diode laser on the disinfection of root canals and in the periapical healing in patients with asymptomatic apical periodontitis, testing an adjuvant laser protocol for root canal retreatment (RCR). The null hypotheses were as follows: (1) there was no difference in the bacterial counts between experimental (RCR with laser) and control (RCR without laser); and 2) there was no difference in the periapical healing between roots treated with laser and the control group.

Materials and methods

Ethical aspects and selection of participants

The protocol was in accordance with the Declaration of Helsinki and was approved by the local Research Ethics Committee (CAAE: 93,708,518.3.0000.5419). The clinical study records were registered in the Clinical Trials Registry (ReBEC: 2.819.612) and informed consent was obtained from all participants.

The inclusion criteria were adults with a single-rooted tooth with post-treatment asymptomatic apical periodontitis for at least 2 years, closed apex, good health, and absence of periodontal disease and abrupt curvatures. The exclusion criteria were pregnant women; fractured, perforated, or resorbed teeth; and patients who had undergone antibiotic treatment during the preceding 6 months [4, 6]. Patient recruitment was conducted from March to September 2019. When a patient had more than one tooth matching the inclusion criteria, only one tooth was selected for the study. The CONSORT flow diagram is shown in Fig. 1.

All patients, examiners, and the statistician were blinded to the type of intervention (laser or placebo) in this randomized and triple-blind trial. A single endodontist performed RCRs and microbiological tests.

Sample size calculation and randomization

The current literature on endodontic retreatment and our dental school records were evaluated to estimate the sample size requirements [3, 6]. Epi Info Software version 6 was used according to confidence intervals. Altogether, a sample size of 30 was deemed adequate, with an additional 10% considering potential patient dropouts. A significance level of 5% was considered. The patients were randomly divided into two groups according to the intervention (laser irradiation [LI] or placebo irradiation [PI]) using an equal allocation rate (<http://www.random.org>).

Root canal filling removal

After primary periapical radiography, antisepsis of the oral cavity was performed with 0.2% chlorhexidine gluconate mouthwash (Periogard; Colgate-Palmolive, New York, USA) for 1 min, followed by local anesthesia (2% lidocaine with adrenaline 1:100,000; DFL, Taquara, Brazil). Dental prophylaxis was performed before the tooth was isolated using a rubber dam. The tooth and the rubber dam were disinfected using 1% iodine tincture (Vic Pharma, Taquaritinga, Brazil). Subsequently, 5% sodium thiosulfate was used to inactivate the disinfectants to avoid interference with microbiological sampling.

The coronal restoration was removed with sterile burs (KG Sorensen, Cotia, Brazil). If necessary, the posts were removed using a piezoelectric ultrasonic device (Profi II Ceramic; Dabi Atlante, Ribeirão Preto, Brazil). The root canal filling material was removed using a combination of ultrasonic tip (Clearsonic; Helse Ultrasonic, Santa Rosa de Viterbo, Brazil) and K-files (Dentsply Maillefer, Ballaigues, Switzerland). No solvent was used, and the canals were irrigated with 5-mL sterile saline solution.

After establishing apical patency, the working length was determined as 0.5 mm short from the apex using an apex locator (Morita Marques, São Paulo, Brazil). The first microbiological sampling was taken as described below. The canal filling removal and the working length were confirmed with digital radiographs.

First microbiological sampling (S1-baseline)

Before and after each microbiological sampling procedure, the weights of three sterile Eppendorf tubes containing three sterile #25 paper points (Tanari, Manacapuru, Brazil) and a size 25 K-file were measured to set the clinical specimen mass. Root canal sampling was performed at three different periods during the treatment: after removal of the filling material (S1-baseline LI or PI), after diode laser or

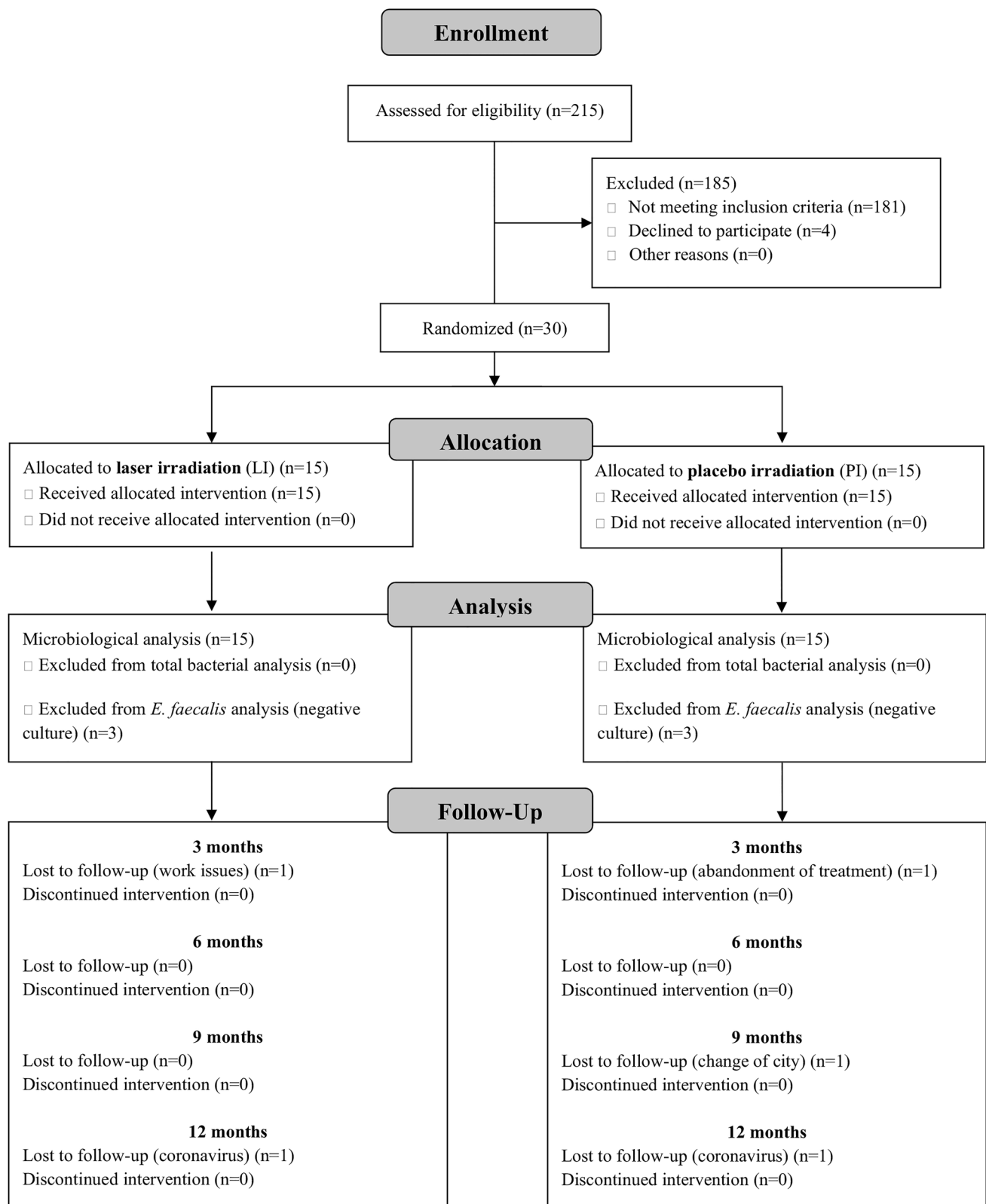


Fig. 1 The CONSORT flow diagram of the clinical trial

placebo irradiation (S2-LI or PI), and after conventional RCR followed by laser irradiation or placebo application (S3-RCR + LI or RCR + PI). Sterility controls were taken at the second visit. All canals were moistened with sterile saline solution before sampling, and the paper points were placed into the full length of the root canal for 60 s to absorb as much fluid as possible. Subsequently, the dentinal bacteria were recovered by moving the K-file three times along the dentinal wall from the apical to the coronal end. All samples were immediately placed inside sterile tubes containing reduced transport fluid (RTF) and promptly transported to the microbiological laboratory. The colony-forming unit (CFU) counts from the pre-disinfection sample served as a baseline for comparison throughout the study.

Laser irradiation and the second microbiological sampling (S2)

The irradiation protocol was divided into LI or PI, as discussed earlier. For the LI protocol, the root canals underwent irradiation with a 980-nm diode laser (Dentsply Sirona, York, USA) with an energy power of 1.5 W at a frequency of 100 Hz in a pulsed mode. The canals were irrigated with 5-mL sterile saline solution, and one sterile absorbent paper point (Tanari, Manacapuru, Brazil) was inserted into the canal and removed after 5 s. The excess moisture of the canal lumen was removed, and humidity remained inside dentine tubules before irradiation. The optical fiber (320 μ m) was introduced 1 mm short of the apex and recessed in helical movements. The laser irradiation was done for 20 s (lasing 4 times of 5 s each, and an interval of 10 s between consecutive movements) [7, 10, 12, 24, 25]. Both the patient and operator wore protective eyewear during laser irradiation. For the PI protocol, the optical fiber was introduced following the same protocol described for LI, but without light emission [17]. The second microbiological sampling (S2) was performed in a manner similar to that used for baseline sampling to analyze the isolated effect of the diode laser in the root canals.

Biomechanical preparation and the third microbiological sampling (S3)

The RCR was performed using a Reciproc R50 instrument (50/0.05; VDW GmbH, Munich, Germany), and the root canals were irrigated with 6 mL of 2.5% NaOCl and 6 mL of 17% EDTA, followed by 6 mL of 2.5% NaOCl under agitation with an ultrasonic tip (Irrisonic; Helse Ultrasonic) for three cycles of 20 s for each solution [26]. To neutralize the NaOCl solution, the canals were irrigated with 5 mL of 5% sodium thiosulfate for 1 min and 5 mL of sterile saline solution (3), followed by the second laser (RCR + LI) or placebo irradiation (RCR + PI). The third microbiological sampling

(S3) was performed following the same protocol used for the previous samplings to assess the effect of laser irradiation combined with biomechanical preparation.

Intracanal medication and root canal obturation

The root canals were dried with sterile absorbent paper points (Tanari, Manacapuru, Brazil), and filled with calcium hydroxide powder (Biodinâmica, Ibiporã, Brazil) mixed with propylene glycol. Coronary sealing was performed with glass ionomer cement (Ketac Molar Easymix; 3 M ESPE, Seefeld, Germany).

After 15 days, the irrigation protocol was performed as described for the first visit and a final flush was done using 5 mL distilled water. The canal was dried with paper points and filled using the lateral compaction technique and AH Plus sealer (Dentsply Sirona). Coronary sealing was performed with glass ionomer cement, and a periapical radiograph was obtained.

Microbiological analyses

After setting the clinical specimen mass and vortexing for 2 min, bacterial suspensions of each sample were diluted in RTF by tenfold serial dilution to 10^3 before plating on culture plates.

Altogether, 50 mL of each dilution was spread onto MM10 SB agar (total counting bacteria) and Enterococcus selective agar (*E. faecalis* counting) using a Drigalsky loop. All MM10 SB agar plates were incubated under anaerobic conditions and all Enterococcus selective agar plates were incubated under aerobic conditions for 7 days at 37 °C.

The number of CFUs per milligram was determined on two replicate plates per sample and statistically analyzed. Microbiological assays were carried out under aseptic conditions in a laminar flow chamber (Quimis, Diadema, Brazil).

Follow-up and periapical bone repair analysis

Clinical and radiographic follow-up examinations were scheduled at 3, 6, 9, and 12 months using an X-ray machine (Dabi Atlante), phosphor plates (VistaScan Mini Plus, Bietigheim-Bissingen, Germany), and a film positioner (Indusbello, Londrina, Brazil) for image standardization. Clinical signs and symptoms were recorded (sensitivity to percussion or palpation, pain, deep periodontal pocket, sinus tract, or swelling) and the restorations were checked. Three trained endodontists blinded to the study objectives and methodology were requested to interpret the radiographs.

The examiners were also calibrated using reference radiographs representing the three scores of radiographic healing: (1) complete healing (presence of a normal periodontal ligament space), (2) incomplete healing (the lesion did

not vary in size or reduced in size without return to normal periodontal ligament space width), and (3) failed healing (pre-existing periapical lesion increased in size). In case of disagreement, the three examiners discussed their findings and a consensus was reached [27].

Data analysis

Demographic data were analyzed using a *t*-test or chi-squared test. CFUs/mg were calculated for each experimental condition and converted into $\log_{10}$. Data analysis was performed using IBM SPSS Statistics version 25.0 (IBM Corp., Armonk, USA) with a significance level of 5%. The Shapiro–Wilk test was used to check the normality of distribution ($P > 0.100$). Post-hoc analysis was performed using Tukey’s test. Data regarding periapical bone repair were analyzed using the Mann–Whitney test. Cohen’s kappa coefficients were calculated to assess intra-observer and inter-observer agreement on radiographic examination (kappa > 0.80).

Results

Demographic data

Root canal sampling was performed on 30 patients (9 men and 21 women, mean age of 55 years). Among these, 15 patients received laser irradiation, while 15 received placebo therapy. Statistical analysis of the clinical features showed no significant differences in sex ($P = 0.371$), age ($P = 0.683$), tooth region ($P = 0.856$), and coronal restoration ($P = 0.239$) between the groups (Table 1).

Microbiological analysis

Total count of viable bacteria

All 30 retreated teeth exhibited positive cultures (CFUs/mg) in the baseline samples (S1) ($n = 30$, fifteen patients per group). Table 2 shows the mean values of CFU/mg expressed in $\log_{10}$ and the standard deviation of each root

Table 1 Demographic and clinical features of the patients in the laser and placebo groups

Patients ($n = 30$)	Placebo irradiation	Laser irradiation	Statistical test	<i>P</i> value*
Gender				
Female	$n = 10$	$n = 11$	Chi square	
Male	$n = 5$	$n = 4$	$\chi^2 = 0.8$	0.371
Age				
G1 (30–55)	$n = 6$	$n = 7$	<i>T</i> test	
G2 (56–80)	$n = 9$	$n = 8$	<i>T</i> value = 0.41	0.683
Mean $\pm$ SD	56.40 ± 8.69	54.27 ± 17.92		
Tooth region				
Maxillary anterior teeth	$n = 5$	$n = 6$	Chi square	0.856
Maxillary premolars	$n = 4$	$n = 1$	$\chi^2 = 8.4$	
Mandibular anterior teeth	$n = 4$	$n = 5$		
Mandibular premolars	$n = 2$	$n = 3$		
Coronal restoration				
Prosthetic crown	$n = 4$	$n = 7$	Chi square	0.239
Composite restoration	$n = 11$	$n = 8$	$\chi^2 = 1.38$	
Preoperative pain	None	None		

* $P > 0.05$ indicated absence of significant difference between the experimental groups

Table 2 Mean values of CFU/mg expressed in $\log_{10}$ and standard deviation of each root canal sampling to the total counting bacteria

Treatment	Sampling procedure		
	S1—baseline	S2—LI or PI	S3—RCR + LI or RCR + PI
Laser irradiation	(2.349 ± 0.736) Bb	(1.352 ± 0.844) Aa	(1.154 ± 1.041) Aa
Placebo irradiation	(2.356 ± 1.034) Bb	(2.395 ± 1.098) Bb	(1.112 ± 0.833) Aa

LI, laser irradiation; PI, placebo irradiation; RCR, root canal retreatment

Different capital letters indicate significant difference between the sampling periods

Different small letters indicate significant difference between the tested treatments

canal sampling in MM10 SB agar; and Fig. 2 illustrates the bacterial reduction percentage after each tested treatment.

Analysis of variance (ANOVA) showed a significant difference in the microbial counts between the laser and the placebo group in the three sampling periods (S1, S2, and S3) ($P=0.000$). In S2, the microbial colony counts reduced considerably (42.44%) after isolated LI ($P<0.05$). This finding differed significantly from the bacterial counts after PI, which exhibited no reduction ($P<0.05$).

After biomechanical preparation (S3), the laser-irradiated group (RCR + LI) exhibited a reduction of 50.87% in the bacterial count with no significant difference compared to the placebo group (RCR + PI), which exhibited a reduction of 52.8% ($P>0.05$). Both placebo and experimental groups showed a significant reduction in bacterial counts after canal preparation (S3) compared to those at baseline (S1).

Enterococcus faecalis count

Twenty-four root canal samples exhibited positive *E. faecalis* cultures (CFUs/mg) at baseline (S1) ($n=24$, twelve patients per group). Table 3 shows the mean values of CFU/mg expressed in $\log_{10}$ and the standard deviation of each root

canal sampling in Enterococcus selective agar; and Fig. 2 illustrates the *E. faecalis* reduction percentage after CFU counting.

ANOVA showed a significant difference in *E. faecalis* count between the treatments (laser and placebo) in all three sampling periods (S1, S2, and S3) ($P=0.042$). In S2, the LI protocol exhibited a reduction of 53.14% in the bacterial count, while PI exhibited a reduction of only 4.85% ($P<0.05$).

After RCR (S3), the laser-irradiated group (RCR + LI) exhibited a reduction of 73.58% in the *E. faecalis* count, which was not significantly different ($P>0.05$) from that exhibited by the placebo group (RCR + PI) (a reduction of 72.18%). The *E. faecalis* counts in the S3 samples of both groups were significantly different from the counts in the baseline samples (S1) ($P<0.05$).

Periapical radiographic healing analysis

Three endodontists were calibrated and blinded to the procedures performed during the radiographic analysis. The intra-examiner kappa coefficients were $A=0.97$, $B=0.86$,

Fig. 2 Mean values of CFU/mg expressed in $\log_{10}$ and the bacterial reduction percentage to each root canal sampling

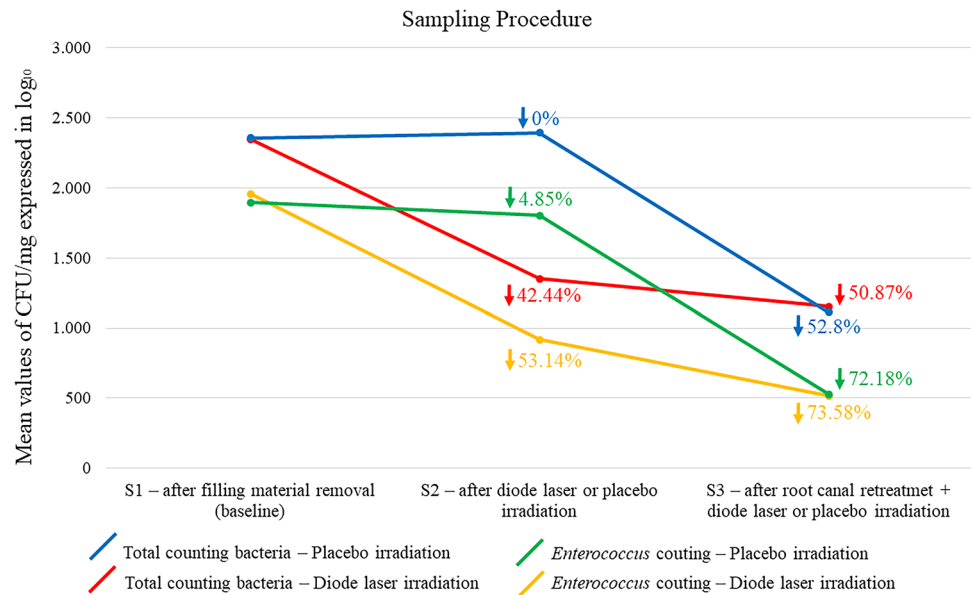


Table 3 Mean values of CFU/mg expressed in $\log_{10}$ and standard deviation of each root canal sampling to the *Enterococcus* counting

Treatment	Sampling procedure		
	S1—baseline	S2—LI or PI	S3—RCR + LI or RCR + PI
Laser irradiation	(1.957 ± 1.219) Bb	(0.917 ± 1.145) Aa	(0.517 ± 0.976) Aa
Placebo irradiation	(1.898 ± 1.186) Bb	(1.806 ± 1.370) Bb	(0.528 ± 0.898) Aa

LI, laser irradiation; PI, placebo irradiation; RCR, root canal retreatment

Different capital letters indicate significant difference between the sampling periods

Different small letters indicate significant difference between the tested treatments

and $C=0.98$. The values for inter-examiner evaluation were $AB=0.84$, $AC=0.96$, and $BC=0.82$.

After 1 year, periapical healing was not significantly different concerning clinical features (sex, age, tooth region, and coronal restoration) ($P>0.05$). Two patients dropped out at the 3-month and 6-month follow-ups with a recall rate of 93.3%. At 3 months, no significant difference was observed between the groups ($P>0.05$), and the healing process had initiated in 89.3% of the lesions, showing incomplete repair (score 2). At 6 months, the laser-irradiated group exhibited better periapical healing than the placebo ($P<0.05$).

At the 9th month, the recall rate was 90% and the difference in the healing process between the groups remained significant ($P<0.05$). After 1 year, the recall rate was 83.3% and the complete healing rates (score 1) were significantly different ($P<0.05$) between the laser (62%) and the placebo group (17%) (Fig. 3).

None of the patients reported changes in their health condition or clinical symptomatology, and there was no tooth loss during the follow-up. Table 4 shows the radiographic

healing scores after RCR and Fig. 4 presents the obtained radiographs of a patient from the placebo group and a patient from the laser-irradiated group.

Discussion

Periapical lesions are inflammatory bacterial infections around the root canal that result in bone resorption and apical radiolucency [1, 27]. Non-surgical and non-invasive RCR should be the first therapeutic option, although this technique frequently has some limitations [1, 2, 28].

Intraradicular disinfection is hampered by the anatomical variability of the root canal system and the multispecies nature of the biofilm [3, 5, 22]. Although *Enterococcus faecalis* is not the only predominant species for RCT failure [29], it can be used as a surrogate endpoint. *Enterococcus faecalis* is a gram-positive coccus frequently isolated from secondary and/or persistent endodontic infections [14] and the lipoteichoic acid on its surface

Fig. 3 Percentage of complete healing cases of each experimental group

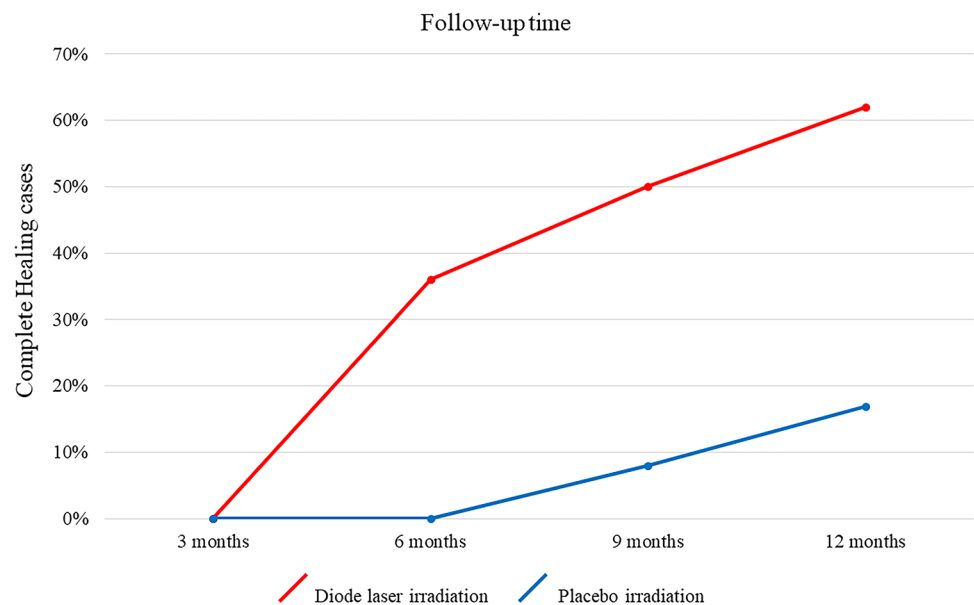


Table 4 Radiographic healing scores after root canal retreatment

	Periapical score	3 m	6 m	9 m	12 m
LI	Complete healing	0/14 (0%)	5/14 (36%)	7/14 (50%)	8/13 (62%)
	Incomplete healing	13/14 (93%)	9/14 (64%)	7/14 (50%)	5/13 (38%)
	Failed healing	1/14 (7%)	0/14 (0%)	0/14 (0%)	0/13 (0%)
PI	Complete healing	0/14 (0%)	0/14 (0%)	1/13 (8%)	2/12 (17%)
	Incomplete healing	12/14 (86%)	13/14 (93%)	11/13 (84%)	9/12 (75%)
	Failed healing	2/14 (14%)	1/14 (7%)	1/13 (8%)	1/12 (8%)
	Mann–Whitney test*	$P=0.577$	$P=0.012$	$P=0.031$	$P=0.027$

* $P>0.05$ indicated absence of significant difference between the experimental groups

LI, laser irradiation; PI, placebo irradiation

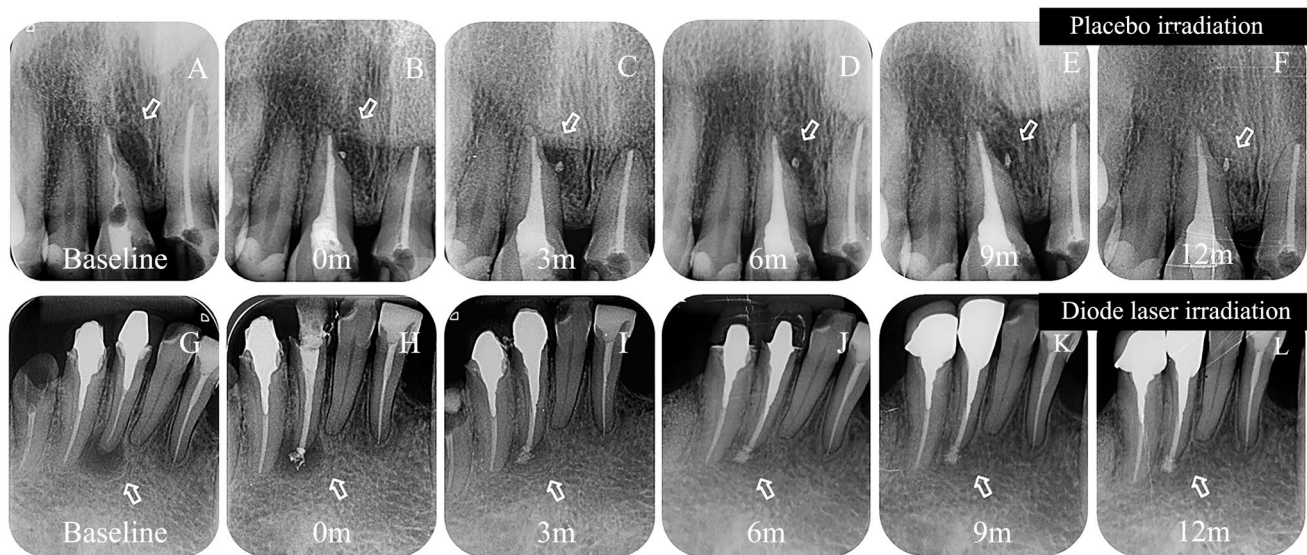


Fig. 4 Radiographic follow-up of periapical status before and after root canal retreatments. Placebo-irradiated tooth: A, before retreatment; B, after retreatment; C, 3 months (score 2); D, 6 months (score 2); E, 9 months (score 2); and F, 12 months (score 1). Laser-irradiated tooth: G, before retreatment; H, after retreatment; I, 3 months

(score 2); J, 6 months (score 1); K, 9 months (score 1); and L, 12 months (score 1). Note that the complete healing of the periapical tissue was achieved in 1 year in the placebo-irradiated tooth and in 6 months in the laser-irradiated tooth

accelerates the release of inflammatory mediators [28]. This bacterial strain was considered in our study due to its virulence, deep tubular penetration, and its high resistance to antimicrobial agents and medications used in RCR, including calcium hydroxide, causing its eradication by conventional means extremely difficult [4, 5, 30].

In addition to RCR protocols, adjuvant techniques such as laser therapy are desirable to improve disinfection. A diode laser at a wavelength of 980 nm was selected in this study due to its simple operation and setup, compactness, affordability, and optical fiber flexibility due to its smaller diameter than other laser devices [11, 12, 18, 23]. This investigation was the first to use a high-power therapy with a 980-nm diode laser in patients needing RCR. The parameters were determined based on previous *in vitro* studies [10–12, 25] to minimize the irradiation risks caused by a temperature increase of the canal surface and surrounding periapical tissues [25].

Despite the less sensitivity, the choice of using a culture-dependent method for the microbiological analysis was because the bacterial strains of interest for this study were not difficult to grow and allowed the detection and counting of CFU/mg. All 30 retreated teeth exhibited positive cultures in the baseline samples for the total viable bacteria count, and twenty-four root canal samples exhibited positive *E. faecalis* cultures. Besides, the microorganisms need to be detectable and viable enough to proliferate and cause treatment failure [30].

Microbiological analysis of the total bacterial counts and *E. faecalis* counts showed an initial antibacterial effect (before the biomechanical preparation) of diode laser on canals, with a 42.44% and 53.14% microbial reduction, respectively. These results are consistent with *in vitro* studies using the same laser equipment [7, 13, 14].

The disinfection mechanism of the diode laser is based on bacterial cell modification or photothermal effect correlated with heat transmission through irradiation [10, 23]. It also depends on the interactions with the target structures [8]. The wavelength of 980 nm in diode lasers has the strongest water absorption as compared to the other wavelengths of diode lasers [31]. The light is well absorbed by water and slightly absorbed by the hydroxyapatite crystals, resulting in light scattering and energy absorption in the dentinal tubule, which is enriched with water [14]. Within ideal parameters, the diode laser disrupts the bacterial cell wall, increasing denatured proteins, cell lysis, and microbial death [32].

Since *E. faecalis* is a non-pigmented bacterium (without endogenous photosensitizers) [33], the action mechanism of 980 nm in diode lasers becomes an advantage therapy over other wavelengths, sparing the need for a dye photosensitizer. Previous studies use dyes (such methylene blue) to stain the bacteria and improve the absorption of laser light (PDT) [3, 6, 20, 21]. However, using these dyes within the root canal is questionable and may have esthetic consequences.

After biomechanical preparation, the reduction in bacterial counts did not differ between the groups. The good disinfection exhibited by both protocols in S3 was related to the shaping and cleaning ability of the flared/tapered files in combination with canal irrigation [34, 35]. Sodium hypochlorite can neutralize endotoxins and dissolve necrotic tissue, depending on the temperature, exposure time, and the methods of irrigation and agitation of the solution [24, 34–36]. Although several irrigation regimens have been suggested, no procedure was standardized yet [26] and none of the tested protocols of this study could completely sterilize the root canal system, according to *in vitro* findings [15].

In addition, there was no significant difference between the bacterial counts after the conventional RCR (S3) and those after isolated LI (S2), suggesting a similar antibacterial effect of chemo-mechanical preparation and diode laser but not synergistic. In this respect, laboratory studies found lower bacterial loads when diode laser was combined with conventional techniques to disinfect root canals [7, 8]. One previous clinical trial [23] evaluated the synergistic effect of 980-nm diode laser and traditional methods of canal preparation and found a successful adjunctive effect. Based on these findings, diode lasers could be an alternative to EDTA or NaOCl in root canal treatment, overcoming the disadvantages such as toxicity during irrigation [13]. They can also be an alternative in open apices, root perforation, and regenerative endodontic procedures due to the cell stimulation effect [9, 21].

RCR and bone repair depend on controlling intraradicular bacterial infection and simultaneous neutralization of unpredictable provocations to the periapical tissue [37]. Bone modulation is a process that involves the activity of osteoclasts, osteoblasts, and osteocytes and is affected by local and systemic health conditions of the patient [1, 20, 37]. Considering that the demographic data analysis showed no differences in sex, age, tooth region, and coronal restoration between the groups, excluding possible bias in the results. However, these factors need to be evaluated in future clinical trials.

To the follow-up analyses, the cone beam computed tomography (CBCT) exams could be a more accurate method for lesion visualization. Nonetheless, this imaging modality has a higher radiation dose and suffers from the inherent presence of artifacts, especially when the tooth of interest is filled with high-density materials such as gutta-percha, endodontic cement, and metal posts, which severely reduces diagnostic accuracy [38, 39]. We choose to use conventional dental radiography, once it seems to have a better risk–benefit for the patients and to be a widely used exam used in dental clinic routine.

In our study, the periapical healing scores from baseline up to 3 months were not significantly different between the two experimental groups. Nevertheless, after 3, 6, 9, and 12 months, an improvement in periapical repair showed that laser irradiation facilitated the healing process over a duration of 1 year.

At the 1-year follow-up, the recall rate was 83.3% (25 patients). The reasons for these losses were probably related to the worldwide coronavirus pandemic and the concerns regarding dental care. The combined proportion of complete healing (score 1) in the laser-irradiated group was also higher (45%) than that in the placebo group in a short-term follow-up. These findings suggest that a 980-nm diode laser can improve the bone formation and, at some point, reduce the follow-up time by accelerating the healed cases. As literature is divergent about the follow-up duration required to define endodontic success, our research will continue the long-term follow-up to compare the complete healing time or the definitive treatment outcome since we commonly observed incomplete wound healing with granulomatous tissue in the periapical region.

Previous studies have reported that a diode laser is a useful tool for bone regeneration and cell proliferation at low power [40, 41]. This fact can be justified by the ability of light to penetrate dental tissues [10, 14]. Upon reaching the periapical area, the laser light modulates several biological processes such as proliferation and differentiation in the cell mitochondria and accelerates bone matrix healing [40, 41]. An important diode laser mechanism of alleviating chronic and postoperative pain involves its ability to decrease the levels of inflammatory mediators and increase neovascularization, which may also accelerate osteogenesis [17, 18, 23].

In this context, the quality of three-dimensional root canal filling providing a seal against bacterial ingress can also influence periapical health by interrupting the communication pathway linking the root canal system and the periapical tissues [27, 28]. As 980-nm diode laser modifies the smear layer and improves the penetration of intracanal dressing/sealer [11, 19], it may indirectly affect the healing process as well.

Calcium hydroxide paste was used in our study to inhibit bacterial growth between visits, as it is the routine first choice in different clinical situations [5, 6]. It is a potent antibacterial and anti-endotoxin agent that has important biological properties [3, 5]. However, the induction of hard tissue deposits and the antibacterial activity of calcium hydroxide dressing depend on the anatomical variability of the canal system and proper material handling [20, 34].

As a clinical trial, some other limitations should be considered. During the periapical bone healing, the host defense factor directly influences the results, and the inclusion of only healthy patients (without significant systemic disease) aimed to minimize this complicating factor. Another limitation is the complexity of root canal morphology, which varies from each patient. To decrease this variability, we selected only single-rooted teeth without abrupt curvatures. Besides the technical difficulty of treatment, we should mention the patient-related challenges that could influence the quality of the root canal treatment. Furthermore, the increase in the chair time can compromise the bacteria viability and affect the research outcome.

After considering the limitations and the difficulty in generalizing the results, we must be careful in assigning the merits of improved periapical bone repair solely to the action of the laser. It is not advisable to establish a direct link between cause and effect, considering the complex biological body healing dynamics. The possibility of an indirect effect of bone repair through root canal disinfection should be reinforced.

Based on our findings, both null hypotheses were rejected. The bacterial counts were lower after laser irradiation when compared with placebo irradiation, showing an antimicrobial effect before the biomechanical preparation. The laser facilitated the periapical repair from 3- to 12-month follow-ups and had 45% more healed cases than the placebo group at 1 year. Future researches could use CBCT for teeth selection, in a way to pair teeth with similar internal anatomy instead of randomizing the cases. Besides, further studies need to be conducted to clarify and detail the disinfection and modulation mechanisms of this promising tool to establish an appropriate therapeutic procedure and greater predictability of RCR outcomes.

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Author contribution L. L. Pelozo: conceptualization, methodology, writing—original draft, investigation and data curation. R.D. Silva-Neto: methodology and data curation. S. Salvador: visualization and resources. M.D. Sousa-Neto: supervision and resources. A.E. Souza-Gabriel: software, validation, formal analysis, writing—review and editing and project administration.

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Declarations

Ethical approval This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Research Ethics Committee of University of São Paulo (CAAE: 93708518.3.0000.5419).

Informed consent The informed consent was obtained from all participants.

Conflict of interest The authors declare no competing interests.

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