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ORIGINAL ARTICLE

The use of optical fiber in endodontic photodynamic therapy. Is it really relevant?

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Abstract This study analyzed the necessity of use of an optical fiber/diffusor when performing antimicrobial photodynamic therapy (PDT) associated with endodontic therapy. Fifty freshly extracted human single-rooted teeth were used. Conventional endodontic treatment was performed using a sequence of ProTaper (Dentsply Maillefer Instruments), the teeth were sterilized, and the canals were contaminated with *Enterococcus faecalis* 3 days' biofilm. The samples were divided into five groups: group 1—ten roots irradiated with a laser tip (area of 0.04 cm^2), group 2—ten roots irradiated with a smaller laser tip (area of 0.028 cm^2), and group 3—ten teeth with the crown, irradiate with the laser tip with 0.04 cm^2 of area. The forth group (G4) followed the same methodology as group 3, but the irradiation was performed with smaller tip (area of 0.028 cm^2) and G5 ten teeth with crown were irradiated using a 200-mm-diameter fiber/diffusor coupled to diode laser. Microbiological samples were taken after accessing the canal, after endodontic therapy, and after PDT. Groups 1 and 2 showed a reduction of two logs (99%), groups 3 and 4 of one log (85% and 97%, respectively), and group 5 of four logs (99.99%). Results suggest that the use of PDT added to endodontic treatment in roots canals infected with *E. faecalis* with the optical fiber/diffusor is better than when the laser light is used directed at the access of cavity.

Keywords Endodontic · *E. faecalis* · Optical fiber · PDT · Root canal

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Introduction

Elimination of the pathogenic microflora from the root canal system during endodontic therapy is one of the main goals of endodontic treatment. Microbial infection plays an important role in the development of necrosis in the dental pulp and the formation of periapical lesions [1]. Infected root canals have a complex microbial flora consisting of cocci, rods, spirochetes, filaments, and fungi that are distributed along the root canal [2, 3] and may exist as loose collections in the moist canal lumen or as dense aggregates (biofilms) adhering to the dentine wall [4]. They may also penetrate the dentine to variable depths, up to 300 μm or more [5]. Eliminating microbial infection from the root canal system to allow healing of the associated periapical lesion is the ultimate goal of root canal treatment.

Contemporary treatment procedures to eliminate the infection include mechanical enlargement of the main canal, irrigation with an antibacterial agent, interappointment dressing of the canal with an antibacterial medicament, and finally, obturation of the resulting space. A range of different techniques result in similar success rates [6], but 2–3% may fail and if retreatments are considered, the

failure rate is even higher [7]. The main causes of treatment failure are the presence of persistent microorganisms, and one of the most common bacteria associated with endodontic failures is *Enterococcus faecalis* [8, 9].

Therefore, it is important that studies be conducted about alternative methods to reduce microbes in endodontic treatment, taking into consideration difficulties such as possibility of microbial resistance/survival after endodontic treatment and the world increase of microbial resistance to antibiotics [10].

A new alternative method for disinfecting root canals is the use of photodynamic therapy [11]. The use of photodynamic therapy may be an alternative as an adjunctive therapy to reduce microorganisms in the endodontic treatment [12]. In this therapy, the light activates a specific photosensitizer that has a lethal effect on microorganisms. The mechanism of the action occurs when the photosensitizer agent absorbs the light source photons and its electrons go into an excited state. In the presence of a substrate, the excited photosensitizer transfers its energy to the substrate, forming short-lived and highly reactive oxygen species, such as the singlet oxygen, causing serious damage to microorganisms through irreversible oxidation of cell components [13].

Several authors had concluded that PDT could be an adjunctive therapy to conventional endodontic treatment to be able to optimize the microbial reduction in the interior of root canals [14, 15]. However, there is not a consensus at the literature about if it is necessary or not the use of an optical fiber/diffusor to achieve better irradiation parameters and better antimicrobial results. Some authors recommend the use, but others not [16]. The aim of this study was to analyze the relevance of use of an optical fiber/diffusor when performing antimicrobial photodynamic therapy (PDT) associated with endodontic therapy and compare the results when the optical fiber/diffusor was not used.

Materials and methods

Preparation of samples

Fifty freshly extracted human single-rooted teeth, with straight canals confirmed by radiographic examination and extracted for periodontal reasons, were collected and stored in sterile saline until employed in the experiment. The canals were enlarged to an apical size of #50 (F5) using ProTaper system (Dentsply Maillefer Instruments SA, Switzerland) and cleaned with 10 ml of 2.5% sodium hypochlorite solution between each endodontic file. The external root surfaces were sealed with two layers of nail polish to avoid environmental contamination. The apical foramen was subsequently closed with composite material (Filtek Z250, 3M, Brazil). In 20 of the teeth, the crowns were removed using a diamond disk, and the roots were shortened to a length of approximately 13 mm. The root canals of all samples were irrigated with 17% EDTA for 2 min followed by irrigation with PBS solution to remove the smear layer. The specimens were sterilized by autoclaving for 15 min at 121°C.

Bacterial strain and growth conditions

E. faecalis (ATCC 29212) were grown in brain heart infusion (BHI) broth at 37°C with shaking (150 rpm) to form a stationary growth phase suspension of 10^9 cells/ml (confirmed by spectroscopy at 540 nm). Ten microliters of this suspension was added into each root canal, and each tooth was placed inside a 1.5-ml microcentrifuge tube that was subsequently sealed, kept upright, and incubated for 72 h at 37°C with shaking (150 rpm) to allow biofilm formation. To facilitate the biofilm formation, the BHI broth was changed every 24 h.

Scanning electron microscopy

One root canal, prepared as described above, was selected for SEM to confirm the biofilm formation. The tooth was split into two halves with a stainless steel chisel. After that, the sample was washed with saline solution to remove the cells non-adhered to the biofilm and then fixed for SEM. The specie were incubated with increasing concentrations of ethanol for 30 min, dried at 37°C for 24 h, and placed on a mounting base. Finally, the samples were coated with gold and examined under a SEM. The microphotographs were obtained at a standard magnification at each third (coronal, middle, and apical) and on the fracture surface.

In vitro experiments

To perform PDT, an initial microbiological sample was obtained using three sterile paper points maintained inside the canals for 1 min to find the initial number of viable microorganisms, and then, the canals were filled with 10 μl of a 60-μM solution of methylene blue (Sigma-Aldrich, USA) and allowed to incubate for 10 min. The samples were separated in five groups and different treatments were performed. On group 1 (G1), all the teeth had the crowns previously removed, and the illumination was performed with the laser tip (area=0.04 cm^2—TwinLaser MMOptics; São Carlos, Brazil) located at the cervical portion of the root, parallel to the root canal lumen. The second group (G2) followed the same methodology as group 1, but the irradiation was performed by the laser with a smaller tip (area=0.028 cm^2—PhotonLase I DMC; São Carlos, Brazil). The third group (G3) consisted of teeth with crown, and the irradiation was performed with the laser tip (area of 0.04 cm^2) at the root entrance as deep as possible on the

pulp chamber. The forth group (G4) followed the same methodology as group 3, but the irradiation was performed by the laser with smaller tip (area of 0.028 cm^2). The final group of teeth with crown (G5) used a 200-mm-diameter fiber/diffusor coupled to diode laser (TwinLaser MMOptics; São Carlos, Brazil). The fiber/diffusor was initially placed in the apical portion (bottom) of the root canal, and spiral movements, from apical to cervical, were manually performed to ensure even diffusion of the light inside the canal lumen. These movements were repeated approximately ten times per minute. All the laser equipment had a wavelength of 660 nm and delivered a total power of 40 mW out of the fiber/diffusor or the tip for irradiation, resulting in a total energy of 9.6 J.

Microbiological analysis

The root canals were irrigated with 1 mL of sterile saline solution to remove the photosensitizer and dried with three sterile paper points (Dentsply Latin America, Petropolis, Brazil), left inside the root canal for 1 min each one. All the three paper points were combined for colony forming unit (CFU) determination. The paper points were placed inside a 1.5-mL microcentrifuge with PBS and vortexed for 30 s. One hundred-microliter aliquots were added to wells of a 96-well plate for serial dilution and streaking on square BHI agar plates for CFU enumeration according to the method of Jett et al. [17]. The plates were incubated for 24 h, and CFU recovered from each treated root canal were calculated.

Reactive oxygen species detection

PDT cause damage to microorganisms through reactive oxygen species, such as the singlet oxygen; the mechanism of the action occurs when the photosensitizer agent absorbs the light, transfers its energy to the substrate, and produces high reactive oxygen species. To quantify the reactive oxygen species generated by PDT with and without the optical fiber/diffusor, the following in vitro experiment was performed.

In a quartz cuvette (1 cm optical path), 3 mL of methylene blue (MB) at 100 μM in distillate water was irradiated with and without the optical fiber/diffusor. The optical density of *N*,*N*-dimethyl-4-notrosoaniline (RNO) at 13.3 μM in the presence of 15-mM L-histidine was analyzed in a spectrophotometer (8453 UV–Visible System, Agilent Technologies, Palo Alto, CA, USA) at 440 nm, after each 30 s of irradiation (energy of 1.2 J for each irradiation) [18]. For the irradiation without the optical fiber/diffusor, the laser tips were positioned over the top of the cuvette parallel to its long axle. For the optical fiber/diffusor, it was inserted approximately until the middle of the cuvette, and spiral movements were performed, simulating the root canal movements.

Light distribution inside the tooth

To analyze the light distribution inside the root canal, especially in the apical region, digital photography of one sample of groups 3, 4, and 5 was evaluated using the software ImageJ (National Institute of Health, USA). A CCD camera was placed orthogonal to the light beam and photographed the intensity distribution of the scattered light. The images were recorded as a bitmap with 32-bit resolution yielding a 256-Gy levels image. Software plug-in transforms the black–white image in a false color image according to the light intensity between values minimum of 0 for no light and 256 for maximum light intensity. The camera captures the scattered light, which is proportional to the local light intensity, and the images correspond to a two-dimensional light intensity distribution model. Therefore, along the laser propagation, it was possible to extract the intensity variation.

Statistical analysis

Median and means for bacterial counts with the corresponding standard deviations were calculated. The mean bacterial counts (in CFU per milliliter) from each group were tested for significant differences by using ANOVA followed by Tukey test. $P<0.05$ was considered statistically significant. Statistical comparisons between means were performed by the software Origin 8.5 (OriginLab, Northampton, MA USA).

Results

The addition of 10 μl of a suspension containing 10^9 cells of *E. faecalis* into the root canal followed by 3 days incubation at 37°C reliably and reproducibly produced biofilm that could be imaged. The presence of a microbial biofilm rather than planktonic bacteria was demonstrated by the failure of irrigation with saline, before the SEM preparation, to remove the bacteria over the tooth tissue (Fig. 1).

The effects of PDT over intracanal biofilm were significant for all the groups; however, the bacterial reduction was superior for optical fiber/diffusor irradiation (G5) and teeth without crowns (G1 and G2). Irradiating the root canal with the laser tip in tooth that has the crown (G3 and G4) resulted in a reduction of less than a log (approximately 85% for the larger laser tip and 97% for the smaller). In teeth without the crown when is possible to irradiate direct over and parallel to the root canal, there were a reduction around 2log (approximately 99%), and with the optical fiber/diffusor even

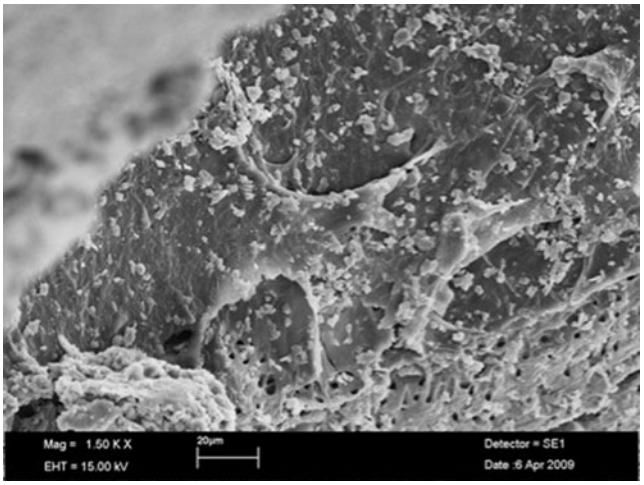

Fig. 1 SEM image of a 3 day-old biofilm on a root canal. The image confirms the presence of a structured biofilm and the increased antimicrobial challenge

when there was a crown, the reduction achieve four logs (99.997%). Figure 2 shows the reduction for the groups.

Figure 3 shows a significant difference in the ROS production in vitro, especially singlet oxygen when the optical fiber/diffusor is used compared with the laser tip. When the fiber/diffusor was moved along the cuvette, the light distribution was more uniform compared with the laser tips static over the photosensitizer solution (data not show).

Figure 4 shows the light distribution along the root when irradiation was performed on groups 3, 4, and 5, and Fig. 5 shows the light intensity through the teeth. The red color representing the maximum intensity clearly confirms that the laser intensity next to the apex is higher when the optical fiber/diffusor is used compared to the others method of irradiation.

Discussion

Previous studies [11, 14–16, 19, 20] showed that a combination of conventional endodontic therapy followed by antimicrobial PDT was effective in reducing bacterial load in ex vivo root canals (for planktonic and biofilm endodontic microorganisms) and in patients. Although the success rate varies from studies and the comparison between them is difficult, these studies have used different photosensitizers, light parameters, and especially different light delivery techniques. In this study, we compared the effect of endodontic PDT using and optical fiber/diffusor and irradiating direct from the laser tip.

Reactive oxygen species, especially singlet oxygen, plays an important role in the damage to microorganisms [10], based on that the quantification of ROS is a valid method to analyze the therapy efficiency. The production of ROS inside a controlled environment showed a significant improvement when the fiber/diffusor was used. Since all the parameters were the same for both groups, the possible explanation for this results could be that when the irradiation was performed with the laser tip (without optical fiber/diffusor), the photoreaction produces ROS, but when most of the oxygen present at the water solution were used, the reaction has a propensity to decrease. On the other hand, when the optical fiber/diffusor was used, the movement inside the liquid allowed the oxygen to diffuse toward the water solution and more molecules of oxygen were available to the photoreaction; also, the light scattering along the cuvette is clearly different for both methods; the light had a better distribution toward the total volume of the container when the fiber/diffusor was used. Fimple et al. [12] even recommend to notch the fiber to produce points of light

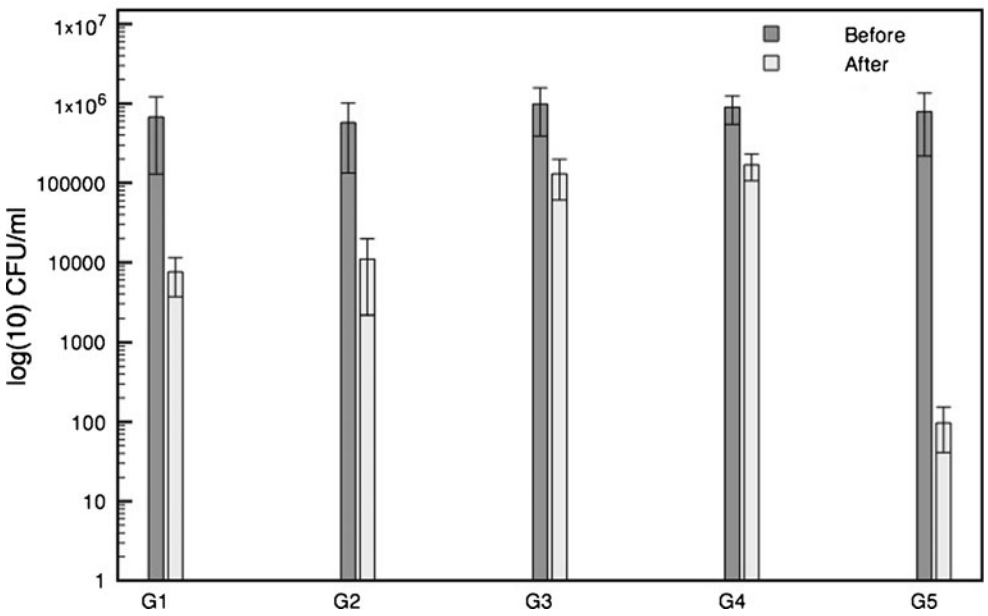

Fig. 2 Log(10) CFU before and after PDT for each group. *G1* teeth without crown irradiated with the larger laser tip, *G2* teeth without crown irradiated with the smaller laser tip, *G3* teeth with crown irradiated with the larger laser tip, *G4* teeth with crown irradiated with the smaller laser tip, *G5* teeth with crown irradiated with the 200-µm fiber/diffuser

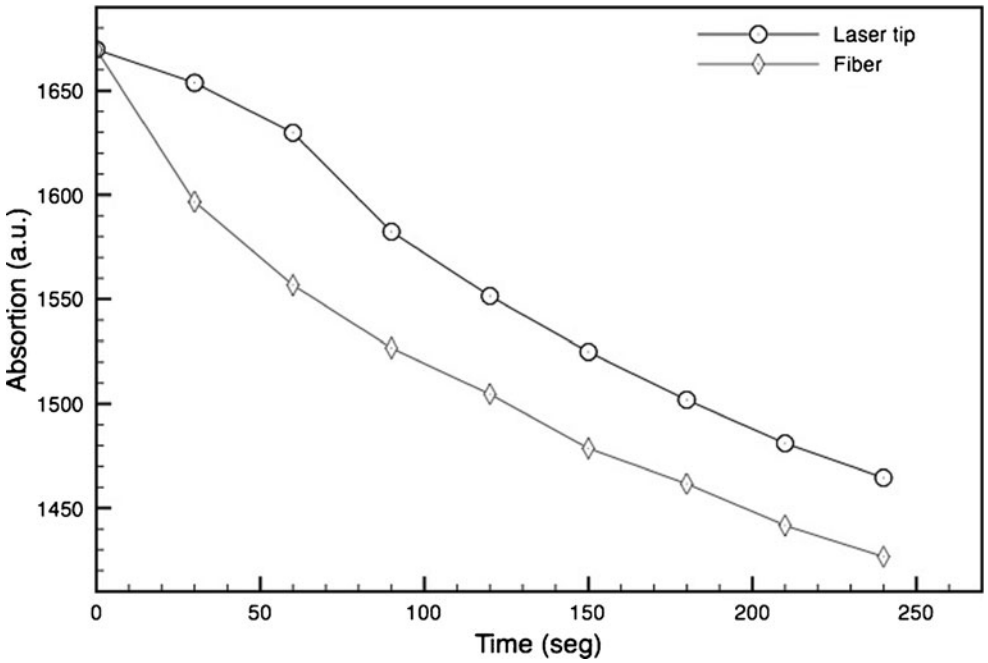

Fig. 3 Oxidation of RNO by ROS produced by irradiation of MB in the presence of histidine to produce colorless products, measured by loss of absorption at 440 nm. Note that a low absorption by the experiment with the fiber indicates an enhanced production of ROS

scattering inside the fiber allowing better light diffusion along the root canal. According to the authors, in this way the light is uniformly distributed over 360°. The optical fiber used in this experiment was constructed to allow light diffusion along the fiber permitting transmission as a real optical fiber and also internal scattering as an optical diffusor. Figures 4c and 5c show the light distribution by the laser optical fiber/diffusor. In a real optical fiber, the light is transmitted by the fiber and the irradiation occurs only in the tip of the fiber, but in an optical diffusor, the light is distributed along the diffusor. Note in the images the uniformity of the light along the root

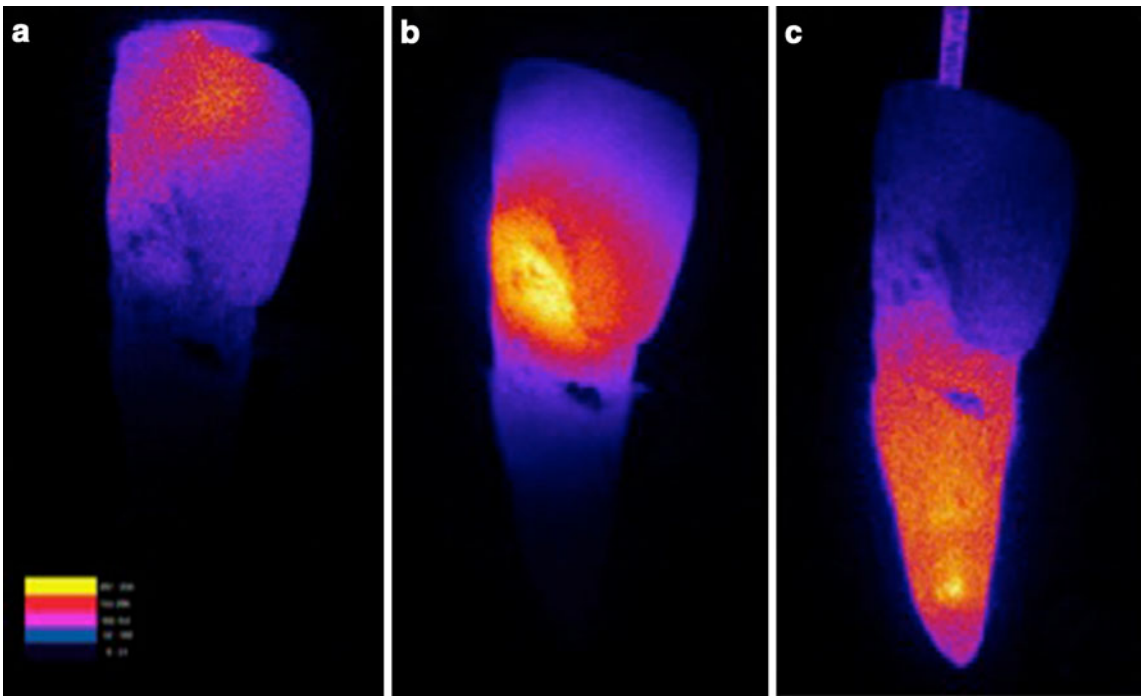

Fig. 4 Representative image of the light scattering intensity of each group. *Image J* software transform the black–white image in a false color image according to the light intensity between values minimum of 0 for no light and 256 for maximum light intensity. **a** G3—irradiation with the larger laser tip, **b** G4—irradiation with the smaller laser tip, and **c** G5—irradiation with the laser optical fiber/diffusor

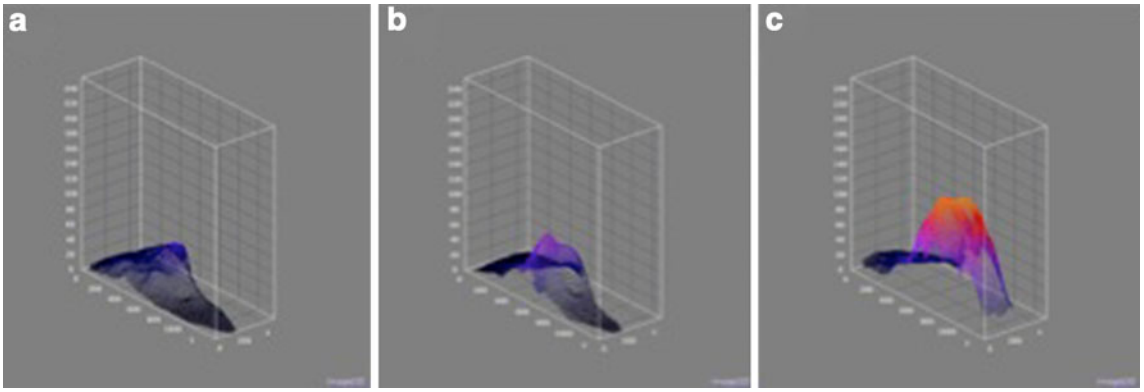

Fig. 5 Histogram analyses of light distribution along the root canal. **a** G3—irradiation with the larger laser tip, **b** G4—irradiation with the smaller laser tip, and **c** G5—irradiation with the laser optical fiber/diffusor. Note that with the use of an optical fiber/diffusor, the light intensity near the apex is greater and the uniformity of the light distribution is better

canal with the use of a fiber compared with the irradiation with the two laser tips.

Regarding with bacterial killing, the presence of an optical fiber/diffusor did notably affect the efficiency of the therapy. The bacterial reduction over intracanal biofilm was significant for all the groups, but the reduction was superior, as we can see in Fig. 2, when the irradiation was performed with the optical fiber/diffusor (G5) and on teeth without crowns by the laser tip (G1 and G2). The photodynamic effect to occur needs the presence of a photosensitizer, a substrate, and a light. The fiber/diffusor allowed the uniform distribution of light along the root canal, and the tips positioned direct over the root canal entrance permitted a similar distribution along the root since the optical anisotropy characteristic of the dentin and the presence of dentine tubules increase the light scattering and propagation beside the teeth tissue [19].

The reduction of less than a log (approximately 85% or 97%), when irradiating the root canal with the laser tip, in tooth that has the crown, could be explained, again, by the optical uniqueness of the enamel and dentine. Added to the characteristics of the dentine cited above, the hydroxyapatite crystals on enamel contribute more significantly to light scattering and reflection on crown, and this decreases the number of photons that could reach the root canal and consequently initiated the photoreaction.

Seal et al. [14] and Lee et al. [20] have reported results using endodontic PDT; both the authors have used phenothiazinium-based PS and low intensity red lasers against Gram-positive bacteria, but did not use an optical fiber/diffusor to access the root canal lumen. Seal et al. concluded that 3% sodium hypochlorite irrigation was more effective against *Streptococcus intermedians* in the endodontic biofilms than PDT with 100 mg/ml toluidine blue and 21 J of 632-nm laser light. Fimple et al. [12] suggest that the use of an optical fiber/diffusor that could uniformly distribute light over 360° can increase the PDT efficiency. Or results in vitro quantifying the ROS production confirms that AND the light distribution along the teeth compared to the CFU recovered from the root canals clearly shows that a better light distribution allow a better results in endodontic PDT. It is missing and between confirming that AND the light distribution.

Conclusion

This study confirms that the use of PDT as an adjuvant to conventional endodontic treatment leads to a significant microbial reduction in root canals biofilms, and for the first time, the relevance of using an optical fiber/diffusor is demonstrated. The light distribution along the root canal is more uniform when the fiber was used or when it is possible to irradiate directly over the canal. In teeth with crown, the irradiation without the fiber/diffusor did not allow a good light distribution inside the root canal, decreasing the PDT efficiency.

A logical conclusion of the present study is that increasing the uniformity of light distribution along the root canal and allowing a better irradiation near to the root apex might lead to greater results in endodontic photodynamic therapy.

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