

Antimicrobial Photodynamic Therapy for the Treatment of Teeth with Apical Periodontitis: A Histopathological Evaluation

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Abstract

Introduction: This study evaluated the *in vivo* response of apical and periapical tissues of dogs' teeth with apical periodontitis after one-session endodontic treatment with and without antimicrobial photodynamic therapy (aPDT). **Methods:** Sixty root canals with experimentally induced apical periodontitis were instrumented and assigned to 4 groups receiving aPDT and root canal filling (RCF) or not: group aPDT+/RCF+ (n = 20): aPDT (photosensitizer phenothiazine chloride at 10 mg/mL for 3 minutes and diode laser [$\lambda = 660$ nm, 60 mW/cm²] for 1 minute) and RCF in the same session; group aPDT+/RCF– (n = 10); group aPDT–/RCF+ (n = 20), and group aPDT–/RCF– (n = 10). Teeth were restored, and the animals were killed after 90 days. Sections from the maxillas and mandibles were stained with hematoxylin-eosin and Mallory trichrome and examined under light microscopy. Descriptive (ie, newly formed apical mineralized tissue, periapical inflammatory infiltrate, apical periodontal ligament thickness, and mineralized tissue resorption) and quantitative (ie, periapical lesion size and number of inflammatory cells) microscopic analysis was performed. Quantitative data were analyzed by the Kruskal-Wallis and Dunn tests ($\alpha = .05$). **Results:** In the aPDT-treated groups, the periapical region was moderately/severely enlarged with no inflammatory cells, moderate neoangiogenesis and fibrogenesis, and the smallest periapical lesions. **Conclusions:** Although apical closure by mineralized tissue deposition was not achieved, the absence of inflammatory cells, moderate neoangiogenesis, and fibrogenesis in the periapical region in the groups treated with aPDT indicate that this can be a promising adjunct therapy to cleaning and shaping procedures in teeth with apical periodontitis undergoing one-session endodontic treatment. (*J Endod* 2012;38:360–366)

Key Words

Antimicrobial photodynamic therapy, apical periodontitis, endodontic treatment

The effective control of bacterial infection in the root canal system is critical for the post-treatment success of endodontic therapy. It has been shown that the endodontic infection in teeth with pulp necrosis and apical periodontitis is of a polymicrobial nature with a high prevalence of anaerobic microorganisms, particularly gram-negative bacteria (1), which ultimately disseminate throughout the root canal system and reach the outer root surface (apical biofilm) (2). The extraradicular infection is inaccessible to the cleaning and shaping procedures, allowing persistence and multiplication of microorganisms and leading to low success rates when no antibacterial medication is used and one-appointment endodontic therapy is performed (3).

Photodynamic therapy (PDT) has emerged as a treatment strategy for eradicating target cells, involving the use of light of a specific wavelength to activate a nontoxic light-sensitive compound (known as photosensitizer) in the presence of oxygen (4, 5). The absorption of photons from the light source by the activated photosensitizer leads it to a triple state of excitation, resulting in energy or electron transfer to available molecular oxygen with consequent formation of highly reactive oxygen species (ROS), such as singlet oxygen and free radicals. This action produces a cascade of oxidative events that ultimately kill microorganisms by causing irreversible damage to essential intracellular molecules including proteins, membrane lipids, and nucleic acids (6). Photodynamic inactivation of microorganisms by local application of photosensitizer and light limits the action of ROS and avoids systemic harmful effects on "friendly" bacterial flora (7). In addition, unlike antibiotics, which have a single target in the microbial cell, ROS generated from the photodynamic reaction has a multifunctional nature and can damage multiple cellular structures, reducing the chances of the development of PDT-resistant bacterial strains (8).

As any treatment modality, antimicrobial PDT (aPDT) should ideally have the capacity to destroy the microorganisms responsible for the disease without causing damage to the host's surrounding healthy tissues. Low toxicity and rapidity of effect are desirable qualities of aPDT (9). It has been established that photosensitizers, which have a strong cationic charge, can rapidly bind and penetrate bacterial cells, and, thus, these compounds show a high degree of selectivity for microorganisms over host cells (10). However, even though studies (11, 12) have concluded that aPDT is less damaging to the host tissues, the concentration used for cytotoxicity assessment in these studies is usually lower than that of bacterial killing. Selectivity toward bacteria

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or mammalian cells is mainly determined by the concentration of the photosensitizer and the time of interaction, and these factors should always be considered when establishing a treatment protocol. Using a root canal model that simulated an *in vivo* response (13) evaluated the cytotoxicity and selectivity of aPDT under *in vitro* and *ex vivo* conditions on fibroblast L929 cells and *Enterococcus faecalis* and found a concentration-dependent bactericidal effect with less destruction of mammalian cells and photokilling of bacteria at a significantly faster rate. However, the results of cell culture studies cannot be directly extrapolated to those obtained in tissues, and, therefore, studies evaluating tissue response to aPDT should be performed.

Low-level lasers are the most frequently used light sources because they are monochromatic in a wavelength specific for activation of the photosensitizer in aPDT (14, 15). A frequently used class of antimicrobial photosensitizers is the blue dyes known as phenothiazinium salts, such as methylene blue (14), which have phototoxic efficiency against a broad range of microorganisms (15) and have been widely used in PDT studies (12, 16–18).

aPDT has been evaluated for photokilling of bacteria in several clinical fields. Specifically in endodontics, aPDT has been used for root canal disinfection as an adjunct to standard endodontic treatment (18–20). However, only the antimicrobial effect of aPDT has been evaluated so far, and there are no studies investigating tissue response to this therapy in teeth with pulp necrosis and periapical lesions. The aim of this study was to evaluate the *in vivo* response of apical and periapical tissues of dogs' teeth with experimentally induced apical periodontitis after one-session endodontic treatment with and without aPDT.

Materials and Methods

All animal procedures conformed to the applicable ethical guidelines and regulations of the university's Animal Research Ethics Committee (process number 011/2009). The present study was based on the protocol recommended by the ISO (ISO standard 7405:1997) (21).

Four 12-month-old mongrel dogs weighing 8 to 10 kg were used. The protocol for the induction of apical periodontitis was the same described by Leonardo et al (2) and Silva et al (22). Briefly, the animals were preanesthetized 15 minutes before the operative procedures and received inhalation anesthesia with isoflurane. After pulp tissue removal, 60 root canals (30 teeth) from the second and third maxillary premolars and the second, third, and fourth mandibular premolars were left exposed to the oral cavity for 7 days to allow microbial contamination. After this period, the coronal accesses were sealed with zinc oxide–eugenol cement with no canal treatment to induce apical periodontitis. Radiographs were taken before the operative procedures and thereafter at 15-day intervals until periapical radiolucencies developed, which usually occurred after 45 days (Fig. 1A, C, E, and G).

After the induction of apical periodontitis, the teeth were isolated with a rubber dam, the operative field was disinfected with 2% chlorhexidine gluconate, and the septic-toxic content of the canals was removed in a pressureless crown-down manner with concomitant 2.5% NaOCl irrigation. Size 15 to 25 K-files (Dentsply Maillefer, Ballaigues, Switzerland) were used sequentially at the radiographic apex under irrigation at each instrument change for debridement and standardization of the apical opening. Then, the canals were instrumented using the Pro-Taper Universal System (Dentsply Maillefer) 1 mm short of the radiographic apex and flushed with 3.6 mL 2.5% NaOCl at each change of instrument according to De Rossi et al (23). Next, the canals were dried with sterile paper points, filled with 14.3% buffered EDTA (pH of 7.4 for 3 minutes), and then flushed with saline and dried with paper points.

Four groups were formed according to the following experimental conditions:

1. Group aPDT+/root canal filling (RCF)+ (10 teeth/20 canals): after cleaning and shaping, the photosensitizer (phenothiazine chloride at 10 mg/mL concentration; HELBO Blue Photosensitizer; Helbo Photodynamic Systems GmbH & Co KG, Grieskirchen, Austria) was applied inside the root canal with an endodontic needle apically to coronally and left to react for 1 minute. After this time, the root canal was rinsed with distilled water to remove the excess, dried with paper points, and irradiated with a laser source. A 660-nm wavelength handheld battery-operated diode laser with 20 mW power output (HELBO Therapielaser, Helbo Photodynamic Systems GmbH & Co KG) was used. The laser was delivered through an 8.5-cm-long flexible fiberoptic tip curved at an angle of 60° with a spot size of 0.06 cm in diameter (HELBO 3D Pocket Probe, Helbo Photodynamic Systems GmbH & Co KG). During irradiation, the fiber was placed within the root canal at its total length. The fiber design permits a 3-dimensional exposure of the area, emitting light at the tip and from the lateral sides, thus leading to even light distribution both vertically and horizontally. The photosensitizer was irradiated as recommended by the manufacturer during 1 minute in a continuous wave mode. The fluence delivered to the photosensitizer was 3.3 J/cm². Canals were irrigated with saline, dried with sterile absorbent paper points, and filled in the same session with AH Plus sealer (Dentsply, De Trey, Konstanz, Germany) and gutta-percha cones. The pulp chambers were cleaned, a restorative glass ionomer cement base was prepared, and the access cavities were restored with silver amalgam.
2. Group aPDT+/RCF– (5 teeth/10 canals): this group received the same treatment described in group aPDT+/RCF+ except that the teeth were restored after aPDT without RCF.
3. Group aPDT–/RCF+ (10 teeth/20 canals): after cleaning and shaping, this group received the same treatment described in group aPDT+/RCF+ except that the teeth were restored after RCF without previous aPDT.
4. Group aPDT–/RCF– (5 teeth/10 canals): after cleaning and shaping, the canals were left empty, aPDT was not performed, and the teeth were restored as described in the other groups. All groups were tested in each animal, and the experimental protocols were performed in alternate quadrants in a randomized manner.

The animals were radiographed (Fig. 1B, D, F, and H) and euthanized after 90 days. The maxillas and mandibles with teeth were dissected and sectioned to obtain individual roots, which were fixed in 10% buffered formalin for 48 hours, demineralized in EDTA, and embedded in paraffin. Sagittal 5- μ m-thick serial sections were obtained, stained with hematoxylin and eosin and Mallory trichrome staining techniques, and examined under light microscopy by an experienced examiner blinded to the groups. A descriptive and quantitative microscopic analysis was performed using an AXIO IMAGER.M1 microscope (Carl Zeiss, Jena, Germany) coupled to an AXIOCAM MRc5 camera (Carl Zeiss).

A description of the characteristics of the apical and periapical region of each group was done according to the following histopathological parameters: newly formed apical mineralized tissue, periapical inflammatory infiltrate, apical periodontal ligament thickness, and mineralized tissue resorption (ie, dentin, cementum, and bone). The periapical lesion size (in mm²) and the number of inflammatory cells in the periapical region were determined in microscopic images of hematoxylin-eosin–stained histological sections using the AXIOVISION REL 4.6 software “Automatic Measurements Program” tool (Carl Zeiss). Quantitative data were



Figure 1. Periapical radiographs of the animals (A, C, E, and G) after the induction of apical periodontitis and (B, D, F, and H) after treatments (before histopathological examination). (A and B) Group aPDT+/RCF+, (C and D) group aPDT+/RCF–, (E and F) group aPDT–/RCF+, and (G and H) group aPDT–/RCF–.

analyzed statistically by the nonparametric Kruskal-Wallis and Dunn tests. The significance level was set at $\alpha = .05$.

Results

Descriptive Microscopic Analysis

Groups aPDT+/RCF+ and aPDT+/RCF–. In both groups, all specimens exhibited nonrepaired areas of resorption in the apical cementum. The apical delta ramifications were widened and exhibited connective tissue free of inflammatory cells. The periapical region was moderately/severely enlarged, with no inflammatory cells and moderate neoangiogenesis and fibrogenesis. The alveolar bone presented large nonrepaired areas of resorption (Figs. 2 and 3).

Group aPDT–/RCF+. The cementum exhibited repaired and nonrepaired areas of resorption and connective tissue with inflammatory cells in the apical delta ramifications. The periodontal ligament space was widened and exhibited a diffuse mixed predominantly mononuclear inflammatory infiltrate, which was more concentrated close to the apical opening. Far from the apical opening, the connective tissue presented edema and fibrogenesis in the initial stage (Fig. 4).

Group aPDT–/RCF–. The apical cementum was irregular and presented extensive nonrepaired areas of resorption. The apical cementum lacunae were widened and contained necrotic tissue remnants and inflammatory cells. The severely widened periodontal ligament space exhibited generalized edema, fibrillar dissociation, areas of necrosis, and a dense mixed predominantly mononuclear inflammatory infiltrate,

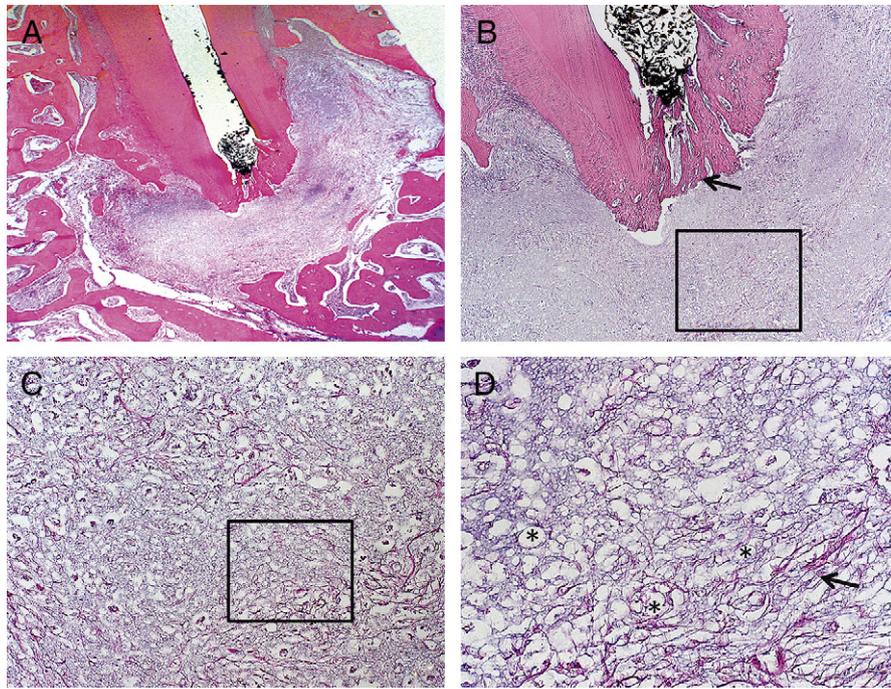


Figure 2. Group aPDT+/RCF+. (A) An overview of the apical and periapical regions (hematoxylin-eosin, Zeiss, $\times 1.25$ objective). (B) The apical and periapical region free of inflammatory cell infiltrate and exhibiting nonrepaired areas of resorption in the apical cementum (*arrow*) (hematoxylin-eosin, Zeiss, $\times 5$ objective). (C and D) A higher magnification of the area demarcated by the rectangle in B and C, respectively, revealing the presence of vessels (*) and fibers (*arrow*) (hematoxylin-eosin, Zeiss, $\times 20$ and 40 objective, respectively).

which either had a focal organization or was diffused throughout the periapical region (Fig. 5). Apical closure by deposition of mineralized tissue was not observed in any of the groups.

Quantitative Microscopic Analysis

The results for periapical lesion size (in mm^2) are summarized in Table 1. There was no statistically significant difference ($P > .05$)

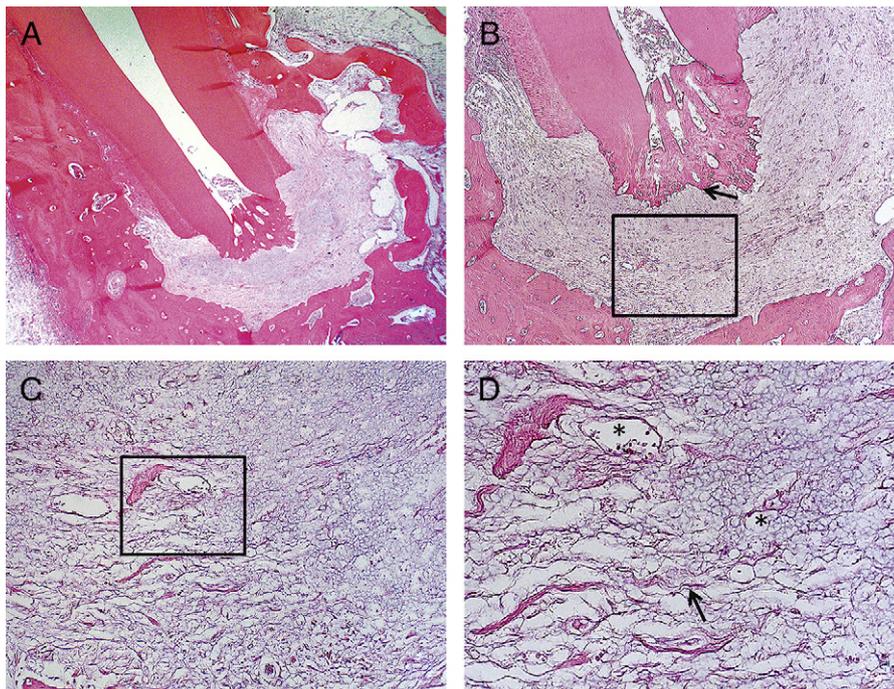


Figure 3. Group aPDT+/RCF-. (A) An overview of the apical and periapical regions (hematoxylin-eosin, Zeiss, $\times 1.25$ objective). (B) The apical and periapical region free of inflammatory cell infiltrate and exhibiting nonrepaired areas of resorption in the apical cementum (*arrow*) (hematoxylin-eosin, Zeiss, $\times 5$ objective). (C and D) A higher magnification of the area demarcated by the rectangle in B and C, respectively, revealing the presence of vessels (*) and fibers (*arrow*) (hematoxylin-eosin, Zeiss, $\times 20$ and $\times 40$ objective, respectively).

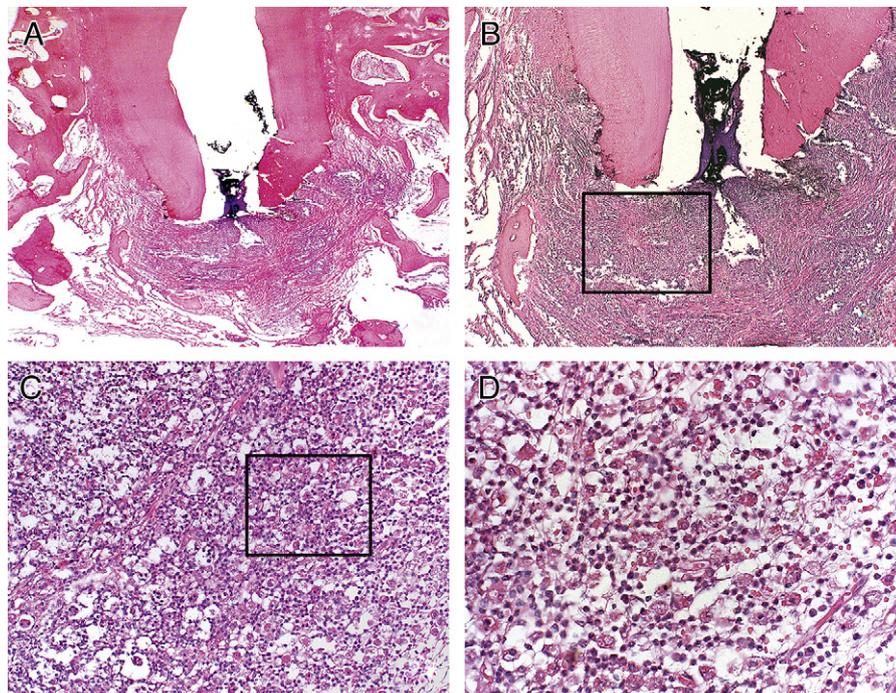


Figure 4. Group aPDT⁻/RCF⁺. (A) An overview of the apical and periapical regions (hematoxylin-eosin, Zeiss, $\times 1.25$ objective). (B) The apical and periapical region showing areas of resorption in the cementum and a more concentrated inflammatory infiltrate close to the apical opening (hematoxylin-eosin, Zeiss, $\times 5$ objective). (C) is a higher magnification of the area demarcated by the rectangle in B showing inflammatory cell infiltrate, generalized edema, and fibrillar dissociation (hematoxylin-eosin, Zeiss, $\times 20$ objective). The rectangular area demarcated in C is magnified in D, showing diffuse mixed predominantly mononuclear inflammatory cell infiltrate and edema (hematoxylin-eosin, Zeiss, $\times 40$ objective).

among groups aPDT⁺/RCF⁺, aPDT⁺/RCF⁻, and aPDT⁻/RCF⁺ or between groups aPDT⁻/RCF⁺ and aPDT⁻/RCF⁻. A significant difference ($P < .05$) was found only between groups aPDT⁺/RCF⁺ and aPDT⁺/RCF⁻ and group aPDT⁻/RCF⁻. The aPDT-treated groups presented the smallest periapical lesions, whereas group aPDT⁻/RCF⁺ presented lesions of intermediate size and the largest lesions were found in group aPDT⁻/RCF⁻.

The results for the number of inflammatory cells in the periapical region are summarized in Table 2. There was no statistically significant difference ($P > .05$) between groups aPDT⁺/RCF⁺ and aPDT⁺/RCF⁻ or between groups aPDT⁻/RCF⁺ and aPDT⁻/RCF⁻. However, the aPDT-treated groups differed significantly ($P < .05$) from the groups without aPDT, which presented a significantly larger number of inflammatory cells.

Discussion

Epidemiological studies have reported that 30% to 50% of root canal treatments fail from residual infections (24–26), which strongly suggests that improved or supplemental endodontic disinfection strategies are needed (27). *In vitro*, *ex vivo*, and *in vivo* studies using aPDT have shown that this approach has the potential to maximize root canal disinfection (18–20, 28–32). However, to the best of the authors' knowledge, there is so far no study evaluating the response of apical and periapical tissues to the use of aPDT in the endodontic treatment of teeth with apical periodontitis (ie, with the presence of tissue infection at the beginning of the treatment). In the present study, the efficacy of aPDT was confirmed by the absence of inflammatory cells and the moderate fibrogenesis and neoangiogenesis in the groups in which aPDT was used. According to Garcia et al (33), neoangiogenesis is seen in most of

the animal models treated with aPDT. Reenstra et al (34) speculated that there must be an increase in oxygen diffusion through the tissues during the application of aPDT, which may favor the repair process because collagen secretion by fibroblasts in extracellular spaces occurs only in the presence of high rates of oxygen pressure. On the other hand, when the root canals were filled without previous aPDT, the apical and periapical region exhibited inflammatory cell infiltrate, fibrillar dissociation, and generalized edema.

An interesting finding of this study was that, although inflammatory cells were not found in the aPDT-treated groups, the healing process was in the initial stage after 90 days. An explanation for this result could be that the photosensitizer may escape into the periapical region and potentially affect the periapical host cells that have taken up the drug. Because of the study goal, all canine teeth had experimentally induced pulp necrosis and apical periodontitis and consequently presented associated periapical bone rarefaction and apical root resorption. In addition, size 15 to 25 K-files were used sequentially at the radiographic apex at each change of instrument during the cleaning and shaping procedures in order to attain apical debridement and standardize the apical opening. Therefore, even considering the application of photosensitizer by a passive syringe inside the root canal, there is a consistent possibility of photosensitizer extrusion and passage of light in the periapex, making the agent toxic to the periapical cells because of light activation.

Although the results of studies evaluating PDT in osteoblast and fibroblast cultures (12, 13), wounds (16), and cutaneous third-degree burns (33) indicate that this therapy causes insignificant deleterious effects to the host's cells, Lambrechts et al (11) found that this therapy may induce damage to fibroblasts, which are the cells involved in wound healing. Another possibility is that the parameters established for the aPDT protocol used in the present study, namely,

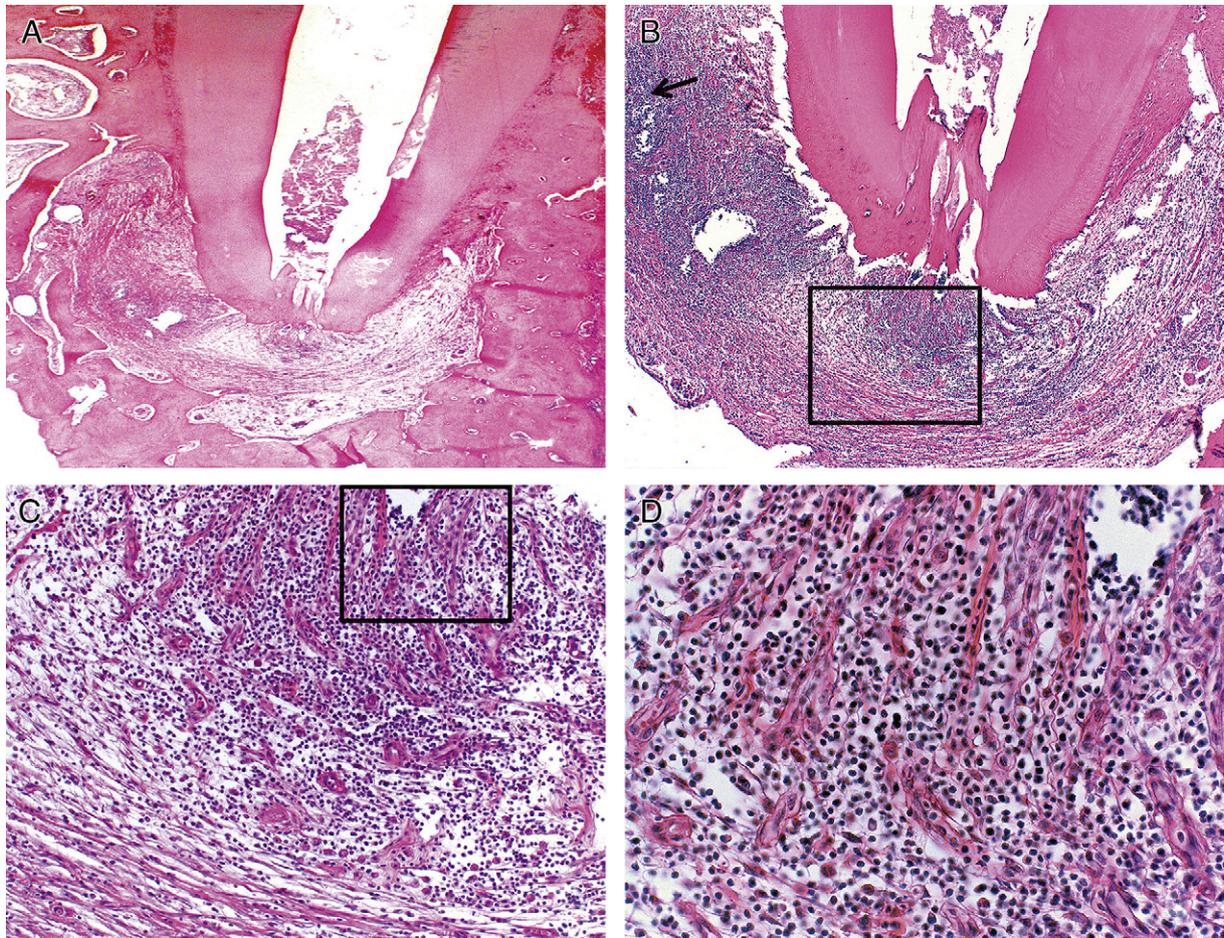


Figure 5. Group aPDT–/RCF–. (A) An overview of the apical and periapical regions (hematoxylin-eosin, Zeiss, $\times 1.25$ objective). (B) The apical and periapical regions showing an intense inflammatory cell infiltrate with focal arrangement (arrow) edema and fibrillar dissociation (hematoxylin-eosin, Zeiss, $\times 5$ objective). (C and D) A higher magnification of the area demarcated by the rectangle in B and C, respectively, showing the presence of dense mixed predominantly mononuclear inflammatory cell infiltrate and edema (hematoxylin-eosin, Zeiss, $\times 20$ and $\times 40$ objective, respectively).

photosensitizer type, concentration and contact time, light dose, and illumination time, were not the ideal for achieving the expected periapical tissue healing. In recent studies, de Oliveira et al (5) and Novaes et al (17) found that aPDT was an effective therapy to treat periodontal disease using the same photosensitizer exposure and irradiation times. It may also be hypothesized that, in the present study, repair of the aPDT-treated groups could have been observed if a longer evaluation period was allowed, considering that at the beginning of the treatment all teeth had apical periodontitis, intense microbial invasion, severe inflammatory process, and active resorption of mineralized tissues.

It is important to establish parameters for the safe use of aPDT under clinical conditions in such a way to promote photodynamic inactivation of microorganisms without being harmful to the healthy tissue cells (11, 12). In their study, Xu et al (12) stated that there is a therapeutic window in which bacteria can be eliminated but the host's healthy cells are left intact. However, this conclusion was reached *in vitro*, and, thus, further *in vivo* studies assessing the tissue response to different parameters of the application of aPDT in endodontics are necessary.

Although in the present study the expected apical closure by deposition of mineralized tissue was not achieved, the absence of inflammatory cells together with the moderate neoangiogenesis and fibrogenesis

TABLE 1. The Periapical Lesion Sizes (in mm²) in the Different Groups

Group	n	Median	Q1-Q3	Min-max	P value*
Group aPDT+/RCF+	20	1356.0	901.0-1938.0	481.0-4619.0	A
Group aPDT+/RCF–	10	1366.0	972.0-2247.0	709.0-3317.0	A
Group aPDT–/RCF+	20	1484.0	1127.7-2160.1	657.3-3243.6	A, B
Group aPDT–/RCF–	10	2729.8	2209.5-3849.8	1612.6-5150.2	B

Group aPDT+/RCF+, aPDT followed by RCF; group aPDT+/RCF–, aPDT without RCF; group aPDT–/RCF+, RCF without previous aPDT; group aPDT–/RCF–, no aPDT and no RCF; Q1, first quartile; Q3, third quartile.

Kruskal-Wallis test: $P = .0024$.

*Different letters indicate a P value $< .05$ for the Dunn post test.

TABLE 2. The Number of Inflammatory Cells (average of 3 measurements) in the Periapical Region

Group	n	Median	Q1-Q3	Min-max	P*
Group aPDT+/RCF+	20	0	0.0-0.0	0.0-0.0	A
Group aPDT+/RCF-	10	0	0.0-0.0	0.0-0.0	A
Group aPDT-/RCF+	20	19947.7	13640.0-22260.0	10409.0-26577.3	B
Group aPDT-/RCF-	10	8425.2	7216.8-12854.8	5005.0-14533.3	B

Group aPDT+/RCF+: aPDT followed by RCF; group aPDT+/RCF-, aPDT without RCF; group aPDT-/RCF+, RCF without previous aPDT; group aPDT-/RCF-, no aPDT and no RCF; Q1, first quartile; Q3, third quartile.

Kruskal-Wallis Test: *P* < .001.

*Different letters indicate a *P* value <.05 for the Dunn post test.

in the periapical region in the aPDT-treated groups indicate that this can be a promising supplemental therapy to biomechanical root canal preparation in teeth with apical periodontitis subjected to one-session endodontic treatment.

Acknowledgments

The authors deny any conflicts of interest related to this study.

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