Molecular Analysis of the Antibacterial Effects of Photodynamic Therapy in Endodontic Surgery: A Case Series

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Abstract

In this case series, bacterial reduction promoted by antimicrobial photodynamic therapy (PDT) used during endodontic surgery was evaluated. Cases were also followed up, and the surgical outcome was reported. The study consisted of 19 teeth with posttreatment apical periodontitis that were consecutively treated by endodontic surgery. After apicoectomy, the root end was treated with PDT using methylene blue as the photosensitizer. Bacteriologic samples were taken from both the cut root surface and the root-end cavity before and after PDT. Samples were analyzed for the total bacterial and *Streptococcus* group counts using quantitative real-time polymerase chain reaction. EndoSequence BC-RRM Putty (Brasseler, Savannah, GA) was used as the root-end filling. Patients were followed up, and the surgical outcome was evaluated. The reduction in bacterial counts after the PDT approach was analyzed using the nonparametric Wilcoxon signed rank test. PDT significantly reduced the total bacterial and streptococcal counts in both root-end cavities and resected root surfaces ($P < .05$). The success rate for 15 teeth that were available for recall after 12 to 21 months was 93% under a loose evaluation criterion and 73% under a rigid one. Used during endodontic surgery, PDT significantly enhanced disinfection of the cut surface area and root-end cavity. Cases treated with PDT showed a high healing rate. *(J Endod 2018;44:1593–1597)*

Key Words

Antimicrobial photodynamic therapy, endodontic surgery, outcome, posttreatment apical periodontitis

Significance

PDT has been widely proposed for the treatment of infectious diseases. When used during endodontic surgery, PDT significantly reduced the counts of total bacteria and *Streptococcus* species in root-end cavities and on resected root surfaces. Teeth treated with PDT exhibited satisfactory outcomes.

Endodontic surgery is an important treatment modality to manage posttreatment apical periodontitis. Surgical procedures usually involve curettage of the inflammatory lesion followed by root-end resection (apicoectomy), root-end preparation, and root-end filling (1). These steps are intended to remove or control the primary causes of posttreatment apical periodontitis (ie, intraradicular or extraradicular infections) (2). Curettage of the lesion removes the extraradicular infection, if present, whereas root-end resection and preparation eliminate extraradicular biofilms and/or bacteria in the very apical part of the canal system, including ramifications. Placement of an adequate root-end filling is expected to entomb residual bacteria in the canal.

The success rate of endodontic surgery may range from approximately 75%–95%, with better results associated with the use of magnification and bioceramic filling materials (3–10). Persistent postsurgical disease is usually caused by infection in the following situations: bacteria located in apical ramifications or isthmuses not cleaned or filled during surgery, bacteria present within dentinal tubules that were exposed by root-end resection, and/or inappropriate sealing promoted by the root-end filling (11, 12). Therefore, disinfecting the resected root surfaces and the root-end cavity has the potential to improve the outcome of endodontic surgery.

Photodynamic therapy (PDT) has become a promising approach to treat diverse infectious conditions (13, 14). It uses light of a specific wavelength to activate a nontoxic photoactive dye (photosensitizer) in the presence of oxygen. Some of the photosensitizers commonly used in PDT include phenothiazine dyes (methylene blue and toluidine blue, phthalocyanine, chlorine, porphyrin, xanthene, and monoterpene) (15). Diode laser systems have been widely used as the light source. Studies have suggested the potential for PDT to improve disinfection during nonsurgical endodontic treatment (16–21).

PDT also has the potential to be used to improve disinfection during endodontic surgical procedures. The approach is expected not to exert significant thermal effects on the periradicular tissues (22) because it uses a low-intensity laser and its primary mechanism of action is based on photochemical reaction, unlike many other laser therapies (23). There is only 1 previous study (24) evaluating the bacterial reduction

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promoted by PDT in endodontic surgery, and the authors concluded that PDT improved disinfection and might influence the surgical outcome. However, in that study, bacterial analysis was based on culture, which has significant limitations, especially related to its low sensitivity and inability to detect many culture-difficult and as-yet-uncultivated bacteria (25). The authors also followed up their cases for 3 years, but no data on the success rate were reported other than a mean reduction in the lesion area as measured on radiographs (24).

The present article consists of a case series in which PDT was used during endodontic surgery. The PDT antibacterial effects were evaluated using a culture-independent molecular microbiology assay to quantify the total bacteria and *Streptococcus* species (quantitative real-time polymerase chain reaction [qPCR]). In addition, the treated teeth were followed up, and the surgical outcome was reported.

Material and Methods

Subject Population

Sixteen patients (10 men and 6 women; mean age = 49 years; range, 33–83 years) who had been consecutively referred for endodontic surgery to the private practice from 1 of the authors (H.S.A.) were included in this report. Surgery was indicated because apical periodontitis persisted unaltered or increased in size after nonsurgical root canal treatment/retreatment performed at least 2 years previously. Thirteen patients contributed with 1 root canal–treated tooth, and the other 3 patients had 2 root canal–treated teeth (n = 19). All teeth but 1 were single rooted. All teeth were asymptomatic and exhibited radiographic evidence of a periapical bone lesion confirmed by cone-beam computed tomographic imaging, which was requested for surgery planning. Fifteen teeth were associated with a sinus tract. The median lesion largest diameter was 7 mm, ranging from 4–18 mm. In addition to exhibiting posttreatment apical periodontitis, the other inclusion criteria were teeth with adequate root canal fillings in terms of length (0–2 mm short of the apex) and homogeneity, teeth with appropriate coronal restoration without exposure of the root canal to the oral cavity, nonsurgical treatment/retreatment performed at least 2 years before, absence of periodontal pockets deeper than 4 mm, patients who did not use antibiotics over the last 3 months, and patients with no significant systemic disease. The protocol was approved by the institutional ethics committee, and informed consent was obtained from all individuals.

Endodontic Surgery and Sample Taking Procedures

Supragingival biofilms were removed by scaling and cleansing with pumice. The oral cavity was rinsed with 0.12% chlorhexidine for 2 minutes, and the surgical area was swabbed with the same solution. After local anesthesia, a full-thickness mucoperiosteal flap was reflected. All surgical steps were performed following strict aseptic measures to avoid contamination of the surgical site with saliva. In cases in which there was no fenestration of the cortical bone, osteotomy was performed to visualize the root apex. The apical periodontitis lesion was curetted and stored in 10% buffered formalin solution for histopathologic examination. Root-end resection was conducted 3 mm short of the root tip at a 0° bevel using a Zekrya FG 28-mm bur (Dentsply Maillefer, Ballaigues, Switzerland) under sterile saline irrigation. Root-end preparation was performed with an ultrasonic tip P1 (Helse, Santa Rosa de Viterbo, SP, Brazil) extended up to 3 mm deep into the canal space. All surgical procedures were performed under high magnification using an operating microscope.

After root-end preparation, an initial bacteriological sample (S1s) was taken from the cut root surface and another one from the root-end cavity walls (S1c) using sterile paper points soaked with Tris-EDTA buffer (10 mmol/K Tris-HCl, 1 mmol/L EDTA, pH = 7.6). Points were scrubbed against the sectioned surface or introduced into the root-end cavity, respectively, and then transferred to cryotubes containing 1 mL Tris-EDTA buffer and immediately frozen at −20°C. Next, an aqueous solution of methylene blue 0.01% Chimiolux (DMC, São Carlos, SP, Brazil) was applied to the cut root surface and the root-end cavity using disposable syringes and 27-G needles. These areas were then irradiated with a diode red laser (λ = 660 nm, P = 40 mW, E = 7.2 J) for 3 minutes (Fig. 1). The laser tip was replaced by an optical fiber (Φ = 200 mm; MMOptics, São Carlos, SP, Brazil) to obtain access to the root-end cavity, and new irradiation was performed for 3 minutes. After rinsing with sterile saline under vigorous aspiration, samples were once again taken from the same regions as described for S1s and S1c (S2s and S2c). Root-end filling with a bioceramic material (EndoSequence BioCeramic Root Repair BC-RRM; Brasseler, Savannah, GA) was placed.

Molecular Microbiology Analysis

DNA from clinical samples was extracted using a QIAamp DNA Mini Kit (Qiagen, Valencia, CA). Levels of the total bacteria and streptococci on the cut surface and root-end cavity were quantified before and after PDT using 16S ribosomal RNA gene-targeted qPCR with Power SYBR Green PCR Master Mix (Thermo Fisher Scientific, Foster City, CA) on an ABI 7500 real-time PCR instrument (Thermo Fisher Scientific). The total reaction volume was 20 μL. Universal (26) or *Streptococcus* group 16S ribosomal RNA-based primers (27) were used. Conditions of the qPCR assay, standard curve construction, controls, and data analyses were as described previously (28). Sensitivity of the qPCR assays was set at 10^1 bacterial cell equivalents. Data are presented as the counts of cell equivalents per sample. All measurements were taken in triplicate for samples, standards, and controls.

Outcome Evaluation

Cases were followed up to evaluate healing. Preoperative, postoperative, and follow-up radiographs were examined independently by 2 blinded evaluators who are experienced endodontists using criteria described previously in the literature (29, 30). If there was disagreement between the evaluators, the opinion of a third endodontist was requested. Radiographic healing classification was as follows:

1. Complete healing: new bone was completely formed, and lamina dura was reconstituted around the root.
2. Incomplete healing: the bone radiolucent area reduced or remained stationary, forming a scar.
3. Uncertain healing: the bone radiolucent area decreased in size and presented a circular shape.
4. Unsatisfactory healing: no reduction or enlargement of the former radiolucency was observed.

According to radiographic and clinical evaluation, the outcome was categorized using rigid or loose criteria as follows:

1. Rigid: radiographic success was defined as either complete or incomplete healing (scar tissue formation) and clinically by the absence of signs and symptoms. Uncertain and unsatisfactory healing were considered as failure.
2. Loose: success was defined as complete, incomplete, or uncertain healing with absence of clinical signs and symptoms. Unsatisfactory healing was considered as failure.

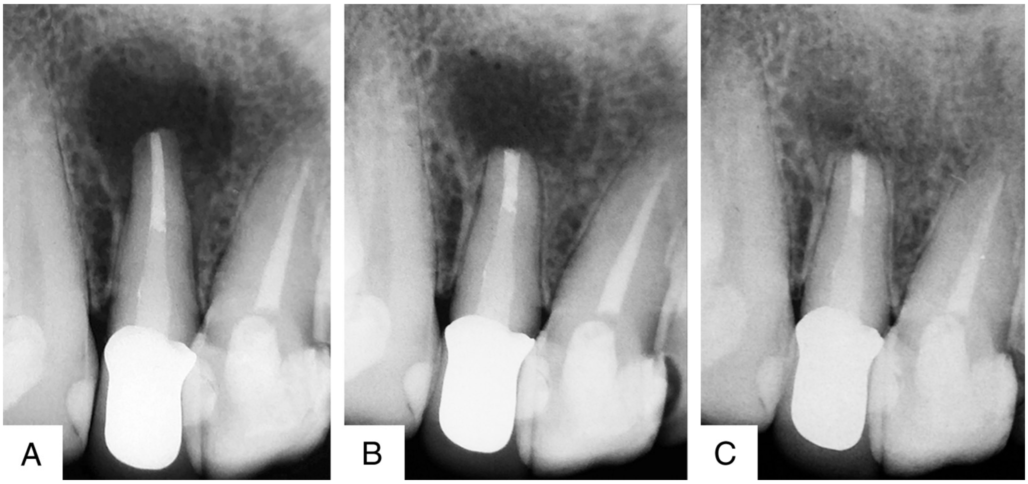

Figure 1. A tooth treated with photodynamic therapy during surgery. (*A*) The preoperative radiograph, (*B*) the postsurgical radiograph, and (*C*) the follow-up radiograph (13 months).

Data Analysis

Quantitative bacteriologic data were expressed as the mean ± standard deviation and median (range) and analyzed using the nonparametric Wilcoxon signed rank test. The significance level was established at 5% ($P < .05$). Qualitative outcome data were expressed as absolute (n) and relative (%) frequencies.

Results

Antibacterial Effects

Molecular microbiology analyses revealed that bacteria occurred in the root-end cavity in 13 teeth before PDT application (S1c), reducing to 9 after PDT application (S2c). The initial samples (S1c) yielded a mean number of 2.71×10^3 (range, 0–2.68×10^4) bacterial cell equivalents, which were substantially reduced in S2c to a mean of 1.64×10^2 (range, 0−8.18×10^2) cells ($P < .001$). The mean bacterial reduction from S1c to S2c was 67.5% (Table 1).

In the cut surface, 15 teeth were positive for bacteria in S1s, reducing to 9 in S2s. An initial mean bacterial load of 3.63×10^2 (range, 0–1.73×10^3) cell equivalents was present in S1s samples, which was also significantly reduced in S2s samples to a mean of 9.23×10^1 (range, 0−6.99×10^2) ($P < .05$). The mean bacterial reduction from S1s to S2s was 70.4% (Table 1).

The effectiveness of PDT against *Streptococcus* species was assessed using a genus-specific 16S ribosomal RNA gene-based approach. Streptococci occurred in 12 samples from root-end cavities and in 14 from the cut surfaces. After PDT, these bacteria were found in 8 samples of each. The quantitative reduction of streptococcal levels was highly significant after using PDT ($P < .01$). The quantitative data of qPCR using primers for the *Streptococcus* group are depicted in Table 2.

Follow-up Evaluation

The median follow-up period was 16 months, ranging from 12–21 months. It was not possible to perform the follow-up examination of 3 patients (4 teeth) because they moved or did not respond to calls via telephone and e-mail. The success rate was 93% (14/15 cases) when the loose criterion was used. Using the rigid criterion, the corresponding figure was 73% (11/15 cases) (Fig. 1).

The only tooth that was categorized as unsatisfactory healing (failure in both criteria) was positive for bacteria in both cut surface and root-end cavity before PDT and still had detectable bacteria in the root-end cavity after PDT. In this case, streptococci were found only in the initial sample from the cut surface. Of the 3 teeth showing uncertain healing (decreasing lesion size), only 1 exhibited bacteria after PDT.

Discussion

This study evaluated the reduction of the total bacterial and streptococcal levels promoted by PDT during endodontic surgery using a quantitative culture-independent molecular approach. Findings showed that PDT induced a significant reduction in bacterial counts both in the root-end cavity and on the cut root surface. These cases were followed up to evaluate the outcome, and a high success rate was observed after 12–21 months, with only 1 tooth showing unsatisfactory healing.

The antibacterial effects observed for PDT are in consonance with numerous previous studies in the endodontic literature (16–21). Bacterial killing effects are mostly related to the transfer of energy from the activated photosensitizer to oxygen in the environment, resulting in the formation of highly reactive oxygen species, such as singlet oxygen and free radicals (15). As for its use in surgery, our

TABLE 1. The Total Bacterial Counts before and after Photodynamic Therapy (PDT) Used during Endodontic Surgery

Location	Before PDT (S1)	After PDT (S2)	Percent reduction
Root-end cavity			
Mean (standard deviation)	2.71E + 03 (6.22E + 3)	1.64E + 02 (2.34E + 02)	67.5
Median (range)	4.21E + 02 (0–2.68E + 04)	0 (0–8.18E + 02)	
Cut root surface			
Mean (standard deviation)	3.63E + 02 (4.39E + 02)	9.23E + 01 (1.74E + 02)	70.4
Median (range)	2.43E + 02 (0–1.73E + 03)	0 (0–6.99E + 02)	

TABLE 2. Streptococci Counts before and after Photodynamic Therapy (PDT) Used during Endodontic Surgery

Location	Before PDT (S1)	After PDT (S2)	Percent reduction
Root-end cavity			
Mean (standard deviation)	1.28E + 03 (3.18E + 3)	1.52E + 02 (2.37E + 02)	64
Median (range)	3.76E + 02 (0–1.38E + 04)	0 (0–7.98E + 02)	
Cut root surface			
Mean (standard deviation)	3.65E + 02 (4.05E + 02)	8.65E + 01 (1.71E + 02)	77.3
Median (range)	2.12E + 02 (0–1.39E + 03)	0 (0–7.17E + 02)	

molecular findings agree with a previous culture study that also found a significant bacterial reduction after PDT (24).

qPCR was used to evaluate bacterial reduction. Culture has been traditionally used for the analysis of antibacterial effects of endodontic procedures, but it presents inherent limitations, such as low sensitivity, especially for the detection of difficult-to-grow anaerobic species, and the inability to grow and identify many culture-resistant species (25). During sample taking, the number of cells collected may fall short of the detection rate with the culture method, and the bacterial presence may be underestimated (31). Molecular methods can detect fewer cells in the sample than culture, and even as-yet-uncultivated bacteria are included in the total bacterial counts (31). Therefore, they provide a more comprehensive analysis of the antibacterial effects of treatment procedures (32).

In addition to the total bacteria, this study also included evaluation of the effects of PDT against *Streptococcus* species because this group has been one of the most prevalent bacteria detected in association with posttreatment apical periodontitis (28, 33–35). Consequently, there is potential risk for streptococci to remain on the cut root surfaces or in the root-end cavities after surgery. Our findings revealed that streptococci were actually very frequently detected in the root-end cavities (63%) and on the cut surfaces (74%) and confirm an association of this bacterial group with posttreatment disease, especially located in the apical part of the root canal system (28).

Diagnosis, treatment planning, surgical procedures, and follow-ups were all performed by the same single operator, an experienced endodontist, reducing variables related to the surgical procedure that might influence the results. All interventions were also performed under high magnification with a surgical operating microscope, with microinstruments and a bioceramic material as root-end filling. The use of magnification and bioceramic materials has been shown to present the best outcomes for endodontic surgery (9, 10). The teeth treated with PDT were followed up for 12 to 21 months, which has been considered as a conclusive period for the large majority of surgical cases (11). Studies have shown that cases that were categorized as success or failure in the first year showed the same healing pattern after longer follow-up periods (3, 36). A high success rate was observed in this study, which was within the range previously reported for endodontic surgery not using PDT (3–10). Whether the PDT approach significantly improves the surgical outcome remains to be determined by further studies comparing the success rates of teeth treated with and without PDT.

Two criteria were used to determine the outcome: a rigid one that considered uncertain healing cases as failures and a lenient one that considered these cases with decreased radiolucencies as success. For instance, the possibility exists that the 3 teeth showing a decreasing lesion size might have healed completely had our follow-up period been extended. It has been observed that the large majority of cases showing decreasing radiolucencies at the 1st or 2nd year of follow-up will eventually heal in the long-term (11). Nevertheless, because in some cases the lesion decrease can halt or even revert and expand again, characterizing a failure in the long-term (36, 37), we decided to report on both criteria.

The significant antibacterial reduction promoted by PDT may make it interesting as a supplementary approach during surgery of teeth with complex anatomy, including those with isthmus or C-shaped canals. In the present study, the large majority of included teeth were single rooted. The only molar treated with PDT showed no bacteria in any of the samples taken, and surgery resulted in success. Because bacteria remaining in anatomic complexities may lead to postsurgical disease (11, 38), future studies should focus on teeth with an increased probability of isthmus or C-shaped canals in order to evaluate the effects of PDT on the surgical outcome.

In conclusion, PDT used during endodontic surgery significantly reduced the levels of total bacteria and streptococci in the root-end cavity and on the cut surface. Cases treated with PDT exhibited a high healing rate.

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

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