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Antimicrobial capacity of photodynamic therapy on apical debris of root canals instrumented with a reciprocating system

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***Abstract*— *Purpose: The aim of this study was to evaluate the antimicrobial action of photodynamic therapy (PDT) on apically extruded debris after root canal system (RCS) instrumentation using WaveOne Gold (WOG) files. Methods: Thirty mesial roots from permanent mandibular molars were selected. The mesiobuccal canal of each root was contaminated with standard-strain Enterococcus faecalis ATCC29212 for 21 days and randomly divided into two groups (n=15): WOG, canals instrumented with WOG file; WOG + PDT, photodynamic therapy performed after instrumentation with WOG. The parameters used for PDT were: low power laser with a wavelength of 660 µm, power of 100 mW, energy of 9.6 J, irradiation time of 96 seconds, and fiber diameter of 600 µm. E. faecalis samples, as well as apical debris, were collected before and after each procedure. Descriptive analysis was performed and the Kruskal-Wallis (Dunn) nonparametric test was applied, with a significance level of 5%. Results: Significant reductions in E. faecalis were observed after instrumentation in both groups ($p<0.05$). Regarding debris, microbial reduction occurred only in the group in which PDT was performed after instrumentation ($p<0.05$). Conclusion: The combination of PDT and reciprocating instrumentation effectively reduced Enterococcus faecalis burden in apically extruded debris.*

Keywords*— *debris, endodontic treatment, enterococcus faecalis, photodynamic therapy.

I. INTRODUCTION

The persistence of microorganisms within the root canal system (RCS) is the main cause of endodontic treatment failure, and is associated with persistent or new-onset periradicular lesions [1,2]. The high prevalence of Enterococcus faecalis in endodontic infection has been attributed to its ability to survive in adverse conditions [3-7].

During mechanical and chemical root-canal preparation, extrusion of material beyond the root apex occurs. The presence of bacteria in this extruded debris may result in postoperative complications such as pain and inflammation, of varying intensity depending on host immune response and virulence of the microorganisms involved. Even when appropriate technique is followed, treatment failures can occur due to the restricted access of instruments and substances to certain regions, which may hinder diffusion of chemical agents into the RCS. New technologies are required to overcome this [3,8 -13].

Photodynamic therapy (PDT) is a treatment modality based on the interaction of low-intensity visible light with a nontoxic photosensitizer in the presence of oxygen, resulting in reactive oxygen species (singlet oxygen and hydroxyl radicals) capable of penetrating microorganism cells. PDT can destroy microorganisms without damage to adjacent tissues and with no risk of bacterial resistance

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[2,14-18]. Several studies have proven the efficacy of PDT in intracanal microbial reduction [1,3,8,10,12,15,19-27].

The aim of this study was to evaluate the antimicrobial action of PDT on apical debris when combined with reciprocating instrumentation. The null hypothesis was that there would be no significant microbial reduction in apically extruded debris with this combination as compared with reciprocating instrumentation alone.

II. METHODS

The present study was approved by the local research ethics committee (protocol no. 2.431.557)

Sample Selection and Preparation

Thirty permanent lower first molars were selected. Sample size was calculated by analysis of variance (ANOVA) for a=0.05 and b=0.80; six repetitions per treatment were deemed necessary. The inclusion criteria were:

- Morphologically similar mesial roots, with independent and formed foramina;

- No history of previous endodontic treatment;

- No canal calcifications or obliterations;

- Absence of internal/external and apical resorption;

- Absence of root caries, lacerations, orcracks;

- Root canals with a master apical file size compatible with a #10 K-file;

- Mesiobuccal canal curvature of 10–20° as determined by Schneider's method [28].

Digital radiographs of all teeth were obtained in the buccolingual direction to evaluate the aforementioned criteria and to determine the degree of mesial root curvature. The selected teeth had their root surfaces scraped with a #13/14 McCall universal curette (Trinity Indústria e Comércio Ltda, São Paulo, Brazil). The specimens were then stored in 0.1% thymol (A Fórmula, Salvador, Brazil) for 24 h for disinfection. The teeth were decoronated near the cementoenamel junction to obtain a standard root length of 13 mm. The orifice of the mesiolingual canal was sealed with light-curing resin (Z350 XT, 3M ESPE, Minnesota, USA) in accordance with standard dental restorative technique [5],and the distal root discarded. To facilitate contamination of the RCS with Enterococcus faecalis, all canals were instrumented manually with a #20 K-file (Dentsply Maillefer, Ballaigues, Switzerland) down to working length [5,14]. The working length was established through the visual method by inserting a #10 K-file (Dentsply Maillefer, Ballaigues, Switzerland) into the root canal and advancing it until the file was visible at the apical foramen under the operating microscope. The instrument was withdrawn and 1 mm subtracted from the measurement thus obtained.

The roots were numbered and attached to the cap of an Eppendorf tube (Eppendorf do Brasil, São Paulo, Brazil), which was used to collect any debris extruded through the foramen after instrumentation. The tubes and their caps with roots attached were then sterilized by the moist heat method in an autoclave (Digitale, BS Equipamentos, Indústria e Comércio Ltda., Varginha, Brazil) at 121°C for 15 min.

The roots were randomly divided (www.random.org.br) into two groups of 15 each. Root canals were contaminated with the ATCC29212 standard strain of E. faecalis, reactivated in sterile Brain Heart Infusion (BHI) broth (Sinergia, Campinas, Brazil), and incubated for 24 h at 37°C in a 5% CO2 atmosphere. The 24-h culture was seeded onto a Petri dish containing BHI agar and incubated for a further 24 h at 37°C in a 5% CO2 atmosphere. Once microbial growth was observed, a suspension was prepared in a tube containing 10 mL sterile normal saline solution and adjusted to match a McFarland turbidity standard of 2 (Probac do Brasil, Produtos Bacteriológicos Ltda, São Paulo, Brazil). In a sterile test tube, 5 mL of the prepared solution was added to 5 mL sterile BHI broth to obtain the final suspension. A 20-µL aliquot of the final suspension was injected into each root canal. Specimens were stored in 24-well cell culture plates (Costar, New York, NY). Sterile cotton pellets moistened in sterile distilled water were added to four wells in each plate to ensure a moist environment. The plate lid was closed and sealed with adhesive tape, and the preparation incubated for 21 days at 37°C in a 5% CO2 atmosphere. Every 2 days, 20 µL sterile BHI broth was added to the root canals and the cotton pellets were replaced with freshly moistened ones. The viability and purity of the microorganisms within the canals were checked weekly by random sampling of two teeth using #15 sterile paper points (Endopoints, Rio de Janeiro, Brazil), which were placed and kept inside the canals for 1 min, seeded onto sterile BHI broth, and incubated for 24 h at 37°C in a 5% CO2 atmosphere. After microbial growth, smears and Gram stains were prepared for morphological and stain-based confirmation of bacterial identification.

After 21 days, baseline microbial count and bacterial viability were checked in both groups by the paper points method described above.Subsequently, instrumentation was performed in each group as follows:

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- WOG: root canal instrumented with WaveOne Gold files (Dentsply Maillefer, Ballaigues, Switzerland) (n=15). The canals were instrumented with a WaveOne Gold reciprocating system using a single 25.07 (primary) file powered by an X-Smart Plus electric motor (Dentsply Maillefer, Ballaigues, Switzerland) in WaveOne Gold mode. Instrumentation always took place with three inward and three outward motions (average amplitude 3 mm), performing wall brushing as the instrument exited the canal, with preparation proceeding preferably by thirds until the working length was reached. The irrigating solution employed during preparation was sterile saline, at a volume of 5 mL per root third (total volume 15mL per canal). Between cycles, the tooth was instrumented to its actual length with a #10 K-file (Dentsply Maillefer, Ballaigues, Switzerland), simulating the clinical protocol. At the end of the instrumentation process, final irrigation was performed with 2 mL of sterile saline.

WOG + PDT: WaveOne Gold instrumentation combined with PDT. The RCS was instrumented with WaveOne Gold files (Dentsply Maillefer, Ballaigues, Switzerland) following the same protocol as described for the WOG group. Subsequently, PDT was performed in every third (cervical, middle, and apical) at 4 mm, 8 mm and 12 mm of working length.

PDT protocol

The root canals were filled with 0.5 ml of photosensitizing solution (0.005% methylene blue, Chimiolux, DMC Group and Aptivalux Bioengenharia Ltda., São Carlos, Brazil), with the aid of a syringe. The pre-irradiation time was 2 minutes. Subsequently, the channel was irradiated with a conical optical fiber tip (DMC Group and Aptivalux Bioengenharia Ltda., São Carlos, Brazil), diameter 600 µm, coupled to a diode laser (Therapy XT; DMC Equipamentos Ltda., São Paulo, Brazil; wavelength 660 nm, power 100 mW). The energy deposited in each third of the root from cervical to apical (4 mm, 8 mm and 12 mm) was 3.2 J, for a total energy of 9.6 J [8,15,20]. Spiral movements were performed for 32 seconds in each third.

Microbial collection after instrumentation was performed by inserting #25 absorbent paper points (Endopoints, Rio de Janeiro, Brazil) into the root canal for 1 minute. Subsequently, each root was irrigated with 1 mL of sterile saline to collect residual debris.

Eppendorf tubes containing the absorbent paper points used for baseline and post-instrumentation microbial sampling, as well as tubes containing suspended debris, were shaken for 30 seconds in a tube shaker (Vortex AD 56; Phoenix, Araraquara, Brazil). Serial dilutions were prepared from this suspension to a concentration of 10-5; then, 0.1-mL aliquots of the suspension and each dilution were seeded onto Petri dishes containing BHI agar incubated in a 5% CO2 atmosphere at 37°C for 24 hours. Subsequently, the number of colony forming units (CFU) per plate was counted and the concentration in CFU/mL calculated.

Statistical Analysis

The results were analyzed in Biostat 4.0 software. The Shapiro-Wilk test rejected the assumption of normality; therefore, a descriptive analysis was performed, and the Kruskal-Wallis (Dunn) nonparametric test was applied with a significance level of 5%.

III. RESULTS

There was a significant reduction in microbial counts after instrumentation in both groups ($p<0.05$). Reductions in microbial burden in extruded debris occurred only in the group undergoing PDT after instrumentation ($p<0.05$, Table 1 and Fig. 1).

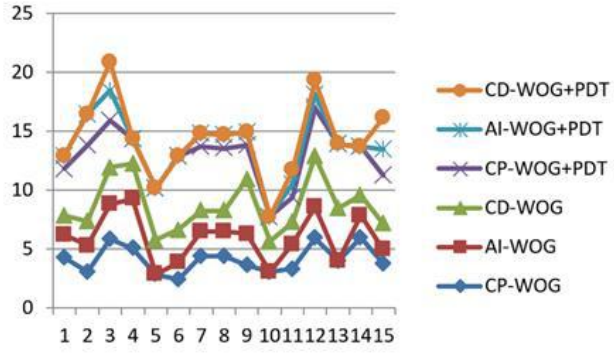

Fig. 1: Microbial count of samples in each group

CD, microbial counts in apical debris; AI, microbial counts in the root canal system after instrumentation; CP, microbial counts in the root canal system at baseline.

Table 1. Medians, interquartile ranges, and Kruskal-Wallis (Dunn) statistic of microbial counts before instrumentation, after instrumentation, and in debris between experimental groups.

Microbial count	WOG	WOG+PDT	(*p*)
Before instrumentation	3.97 (1.51)^{a,1}	4.12 (2.60)^{a,1}	$p > 0.05$
After instrumentation	2.12 (1.17)^{b,1}	1.14 (1.14)^{b,1}	$p > 0.05$
In debris	2.60	0.00	$p < 0.05$

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	$(1.27)^{ab,1}$	$(0.57)^{b,2}$
(p)	<0.05	<0.05

WOG. Wave One Gold instrumentation; WOG + PDT, WOG instrumentation and photodynamic therapy.

Different lower-case letters along the same column and different numbers along the same row denote p <0.05.

IV. DISCUSSION

E. faecalis is considered an opportunistic pathogen. It is strongly associated with persistence of root-canal infection and failure of endodontic treatment [7,17,26,29-32]. In an attempt to reproduce the real-world clinical situation of persistent intracanal contamination, we selected E. faecalis for this experiment due to its ability to penetrate the dentinal tubules and known resistance to antimicrobial agents [15,16,25,27,33-35]. E. faecalis was cultivated for 21 days, sufficient time for formation of a mature biofilm [5,10,27,36,37], which tends to be associated with even greater resistance to the action of antimicrobial agents and PDT due to the high cell density and presence of an extracellular matrix which blocks drug diffusion and absorption [38].

Pre-enlargement of the root canals was performed manually with #20 K-files in order to standardize the initial diameter, facilitate bacterial contamination,and allow bacteria to reach the dentinal tubules [5,25,32,36].

All instrumentation techniques cause extrusion of material beyond the apical foramen, but studies have shown that WOG files extrude less debris [31,37,39-41]. In the present study, the canals were prepared by thirds, always in a smooth crown-down fashion, in order to avoid apical extrusion. Sterile saline solution was used as an irrigant to avoid interference of agents with antimicrobial activity on the results [5,37].

Although chemical-mechanical debridement of the RCS is effective, adequate preparation of areas that are difficult to reach may be impossible due to anatomical complexity and microbial resistance [12,32]. New approaches have been researched to reduce the incidence of biofilm on the infected root canal wall; in this context, several studies have proven the efficacy of PDT as an aid in RCS disinfection [1,3,5,8,10,12,15,16,19,20,22-27,38].

The most commonly used light sources are low-power lasers, which have a specific wavelength for photosensitizer activation that, in addition to having an antimicrobial effect, facilitates rapid repair of periapical tissues and reduces post-instrumentation discomfort [4,6,9,15,38]. The photosensitizer used in this study was methylene blue, due to its ability to absorb light at the wavelength emitted by 660 nm laser, as demonstrated in previous studies [2,5,14-17,33,35,36,38]. The pre-irradiation time used was 2 minutes, which is sufficient to allow the dye to accumulate in the target tissues but not so long as to allow its effect to decline before light application [8,14,20,25,33,38]. Pre-irradiation time is important because Gram-positive bacteria (such as E. faecalis) have a porous outer membrane, allowing faster diffusion of the photosensitizer into the cell [15,16].

Laser was applied within the root canal with the aid of a fiberoptic tip. In most studies, the irradiation protocol involves spiral motions of the fiber, starting from the apical region and moving towards the cervical direction [8,14,15,17,20,25,33,38]. There are no previous studies reporting PDT application by thirds, from cervical to apical, as performed in this study. Several studies have confirmed the efficacy of PDT in intracanal microbial reduction after instrumentation or in combination with irrigating substances, despite the wide range of PDT protocols adopted [1,2,5,6,16,17,20,22,27,33-35,38].

There was a significant difference in microbial count in extruded debris between the WOG and WOG + PDT groups, in favor of the latter. This result suggests that the photosensitizing agent penetrated the dentinal tubules and hard-to-reach areas when activated by the laser, corroborating the findings of Ramalho et al. [9] Use of a fiberoptic tip for intracanal irradiation is an important aid in PDT because it promotes a homogeneous distribution of light along the root canal, allowing the irradiation beam to reach the full length of the RCS and the root apex [2,5,12,15,20,27,33,34,38]; this facilitates greater formation of reactive oxygen species within the dentinal tubules, which enhances microbial reduction [6]. Sabino et al. [38] suggested that the spiral movement of the fiber inside the canal may contribute to reoxygenation of the photosensitizer, allowing a greater formation of reactive oxygen species; in addition, spiral movements can help displace the biofilm and facilitate dye diffusion into deeper dentinal tubules, thus improving PDT efficacy.

PDT was applied by thirds until the working length was reached, a procedure that, to the best of our knowledge, had not been adopted in previous research, and which may have contributed to the reduction of bacteria in the apical debris.

The presence of microorganisms in apical debris may result in worsening of periapical inflammation, causing postoperative pain and flare-ups, hindering the healing process in periapical tissues, and negatively affecting the clinical outcome of endodontic treatment [37,40,41].

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V. CONCLUSION

We conclude that adjunctive use of PDT after reciprocating instrumentation of the root canal system reduced bacterial burden in debris extruded beyond the root apex. This finding provides new perspectives in the field of endodontics.

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