Original Research
Endodontics

Antimicrobial efficacy of the EndoVac system plus PDT against intracanal *Candida albicans*: an ex *vivo* study

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Abstract: This study evaluated the *ex vivo* antimicrobial efficacy of the EndoVac system and the photodynamic therapy (PDT) associated with chemomechanical debridement (CMD) and intracanal medication on *Candida albicans*. Seventy-eight sterile premolars were contaminated with *C. albicans* (ATCC 21433) for 30 days. The teeth were randomly assigned into four groups: Control (CMD with conventional irrigation); Endovac (CMD with EndoVac system); PDT (CMD with conventional irrigation and PDT); and Endovac + PDT (CMD with EndoVac and PDT). After the therapies, intracanal dressing (calcium hydroxide) was applied to all teeth for seven days. Samples were obtained before (T1) and after the therapeutic procedures (T2), and after intracanal medication (T3), plated onto BHI agar and incubated (37°C, 48 h) to determine the colony-forming units (CFU)/mL. The overall mean level of *C. albicans* at baseline was relatively high ($1.85 \times 106 \pm 2.7 \times 106$ CFU mL^{-1}). A significant reduction of *C. albicans* ($p < 0.05$) was observed over time (T1 to T2 and T1 to T3) in all groups. An additional significant reduction from T2 to T3 was observed only in the Endovac group ($p < 0.05$). No differences in mean reduction of *C. albicans* were observed among groups. However, the Endovac group presented the lowest mean counts of *C. albicans* at T3, whereas the PDT group had the highest counts of this microorganism ($p < 0.05$). The EndoVac system of irrigation/aspiration associated with CMD was the most effective therapeutic protocol for reducing intracanal levels of *C. albicans*. PDT showed a very limited efficacy against this species.

Keywords: Root Canal Therapy; Photochemotherapy; *Candida Albicans*; Calcium Hydroxide; Sodium Hypochlorite.

Introduction

Persistent apical periodontitis is generally a result of incomplete elimination of microorganisms from the root canal system.[1,2,3] Several factors have been associated with unfavorable outcomes following root canal therapy, such as resistance of microorganisms to root canal treatment and intracanal medication, anatomical complexities of the root canal, deep invasion of microorganisms into dentinal tubules, and biofilm formation.[3,4,5,6] *Candida albicans* has been shown to be the major fungal species present in root-filled teeth with periradicular lesions, being usually isolated in association with facultative Gram-negative and

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positive bacteria.[2,4,6] In fact, detection of *C. albicans* from root canal infection may have been overlooked due to technical limitations such as the low number of cells for recovery by cultural methods, the use of non-selective media, and the fact that these species are regarded as contaminants because of their colony morphology.[4] *C. albicans* has the potential to adapt to a wide range of extreme environmental conditions due to its capability to survive under anaerobic and static conditions, to penetrate dentinal tubules, to resist intracanal medication, and to form biofilm on the surface of both organic and inorganic material, among others.[4,5,7]

In general, no therapeutic protocol, technique, or specific instrument is able to efficiently shape and completely eliminate microorganisms inside the root canal system.[1] Since persistent endodontic infections have a lower rate of treatment success, combination of conventional treatment and new adjunctive therapeutic strategies could be more effective in reducing or eliminating microorganisms, thus improving the rate of endodontic treatment success.[3] For instance, the EndoVac irrigation/aspiration system (Discus Dental, Culver City, USA) and low-power laser used in photodynamic therapy (PDT) have been introduced into standard therapies to improve root canal disinfection.

The antimicrobial activity of chemomechanical debridement (CMD) is essentially based on sodium hypochlorite (NaOCl) irrigation combined with mechanical debridement.[8] The irrigation solution must be properly delivered to allow it to reach the apical area without forcing the solution into the periapical area. The EndoVac system was developed to make this possible. It is based on an apical negative pressure irrigation/aspiration system, which enables the irrigation solution to reach the canal on its full working length (WL) without the risk of overflow into the periapical tissues.[9,10,11]

As an adjunct to conventional endodontic therapy, PDT has been used to improve disinfection of root canals after CMD.[12,13,14] PDT has a two-step procedure, with the introduction of a non-toxic photosensitizing agent and a light source.[12,14,15] The action of the light on the photosensitizer leads to the production of highly reactive and cytotoxic oxygen species and to consequent injury and death of microorganisms that either have selectively taken up the photosensitizer or have been locally exposed to light.[12]

Thus, the purpose of this study was to evaluate the *ex vivo* effectiveness of the EndoVac system and PDT as adjuncts to CMD associated with intracanal calcium hydroxide [$Ca(OH)_2$] medication in the reduction of *C. albicans* in the root canal system.

Methodology

Microbial contamination

The study protocol was approved by the Human Research Ethics Committee of the Institute for Studies in Public Health of the *Universidade Federal do Rio de Janeiro* - UFRJ (protocol no. 49/2011). Seventy-eight human mandibular premolars were used. Root canals were accessed conventionally and prepared to a standard length of 20.0 mm, and apical patency was established at 20.5 mm.[15] The presence of a single canal was determined by radiographs. To standardize the diameter of apical constriction, root canals were instrumented up to a size 20 K-file (Dentsply Maillefer, Ballaigues, Switzerland). The teeth were dried and the root surfaces were covered with two layers of nail varnish.[15] The teeth were then placed into flasks containing 40 mL of brain heart infusion (BHI) broth (BD, Sparks, USA) and autoclaved at 121°C for 15-20 minutes. *C. albicans* (ATCC 21433) suspensions grown in BHI broth (BD) were adjusted to 1.5×10^8 colony-forming units (CFU)/mL^{-1}, and 1.6 mL was inoculated into the flasks (final concentration of 6×10^6 CFU mL^{-1}). The flasks were incubated at 37°C for 30 days, and the medium was replaced every week. To confirm *C. albicans* purity, Gram staining was performed every week and before the first sample. The tooth surfaces were decontaminated, and the foramen sealed with epoy resin.[15,16] The teeth were mounted vertically up to the cementoenamel junction in blocks made of silicone impression material.[16]

Treatment groups

The teeth were randomly distributed into four groups: Control (n = 20), CMD + conventional irrigation with 5.25% NaOCl; Endovac (n = 19), CMD + irrigation with the EndoVac system (Discus Dental, Culver

City, USA); PDT (n = 20), CMD + conventional irrigation + PDT; and Endovac + PDT (n = 19), CMD + EndoVac system (Discus Dental) + PDT. After the procedures, intracanal medication consisting of $Ca(OH)_2$ paste in 0.85% saline solution (1:1) was used in all teeth from the four groups.

Instrumentation and irrigation techniques

The instrumentation sequence in all groups was performed using ProTaper rotary files (Dentsply Maillefer, Ballaigues, Switzerland) according to the manufacturer's instructions. Files S1 and S2 were used for the coronal and middle sections of the canal (WL = 16 mm), and files S1 and S2, followed by files F1, F2, F3 and F4, were used for the apical section (WL = 20 mm). To ensure patency, recapitulation to WL was accomplished after each rotary instrument with a size 10 file. Every group received the same volume (±21 mL) of irrigation solution (5.25% NaOCl). Each root canal was irrigated with 5 mL of NaOCl before treatment and with 1 mL of the irrigant afterwards. For the final irrigation, the canals were rinsed with 6 mL of 5.25% NaOCl for 60 s, 5 mL of 17% EDTA for 3 min, and 3 mL of 5.25% NaOCl. Conventional irrigation was performed using a NaviTip 30-gauge needle (Ultradent, South Jordan, USA) adapted to a plastic syringe. The needle was placed into the canal without binding and within 3 mm from the working length. In the EndoVac system (Discus Dental), the root canal was initially irrigated with the master delivery tip. After apical preparation, irrigation was performed with the master tip by injecting 3 mL of 5.25% NaOCl and by aspirating it with the macrocannula. Irrigation with the master delivery tip and aspiration with the microcannula consisted of the following: 3 mL of 5.25% NaOCl, 5 mL of 17% EDTA for 3 min, and 3 mL of 5.25% NaOCl.[15]

Photodynamic therapy

The root canals were rinsed with 1 mL of 5% sodium thiosulfate for 1 min and 1 mL of 0.85% saline solution, and dried with absorbent paper points (Endopoints, Manacapuru, Brazil). Subsequently, 0.5 mL of 25 $\mu g\ mL^{-1}$ (67 µmol/L) methylene blue was injected and left for 5 min for pre-irradiation time.[15] The canal was then irradiated by a low-power diode laser (Twin laser, MMOptics, São Carlos, Brazil) through a disposable optical fiber (Twin laser, MMOptics, São Carlos, Brazil) into the root canal along the WL. The fiber was placed in the apical portion of the root canal, 1 mm from the working length, and spiral movements were manually performed from the apical to the cervical direction. The light was applied for 5 min with an output power of 1 W and central wavelength of 660 nm, at a power of 40 mW without the optical fiber (300 J/cm^2 delivery irradiance per treatment). The root canal was again flushed with 10 mL of sterile 0.85% saline solution to remove the photosensitizer and dried with paper points (Endopoints).[15]

Intracanal medication

After all therapeutic protocols, the root canals were dried with paper points (Endopoints), filled with fresh BHI broth (BD) and incubated at 37°C for 48 h in a wet chamber. The broth was then removed and an intracanal dressing ($Ca(OH)_2$ in 0.85% saline solution) was introduced. The teeth were incubated at 37°C for 7 days. The medication was removed with 0.85% saline solution as irrigant.[15]

Sample collection

Before sampling, all root canals were rinsed with 1 mL of 5% sodium thiosulfate for 1 min and 1 mL of 0.85% saline solution. Sampling was performed at the initial contamination with *C. albicans* (T1), 48 h after treatment (T2), and seven days after intracanal medication (T3).[16] The root canals were filled with 0.85% saline solution, and each sample was collected by three paper points (Endopoints) to the WL, for 1 min each.[16] The paper points (Endopoints) were transferred to tubes containing 1 mL of 0.85% saline solution and agitated thoroughly for 5 s. Aliquots of 0.1 mL were plated in triplicate onto BHI (BD) agar plates and incubated aerobically at 37°C for 48 h. The CFU were computed for each sample and the mean of the triplicates calculated.

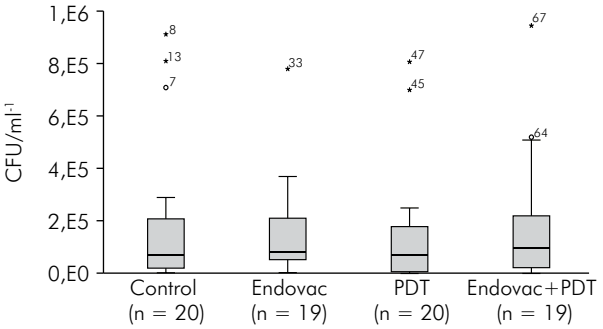
Statistical analysis

A statistical program (SPSS, Statistical Package for the Social Sciences 19.0, IBM Brasil, São Paulo, Brazil) was used for all analyses. Normality distribution of

the variable mean CFU mL^{-1} of *C. albicans* was verified using the Kolmogorov-Smirnov test. Since this variable did not present a normal distribution in this sample, comparisons among groups were performed by non-parametric tests. Significant differences in mean CFU mL^{-1} of *C. albicans* amongst groups were assessed by the Kruskal–Wallis test at each sampling time (T1, T2 and T3). Differences between pairs of groups were examined by the Mann-Whitney test. Changes in mean counts (CFU mL^{-1}) over time (T1 to T3) were analyzed by the Friedman and the Wilcoxon Signed Rank tests. The significance level was set at 5%.

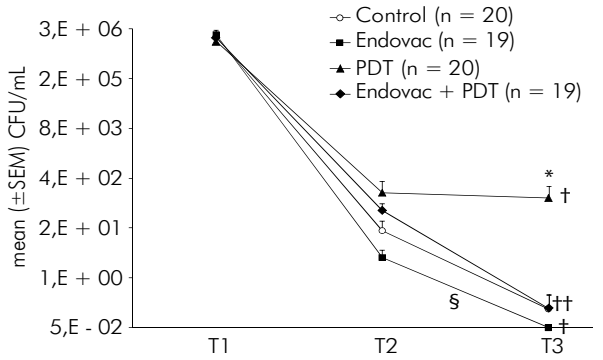
Results

The distribution of the baseline (T1) mean counts of *C. albicans* in the teeth of the four therapeutic groups is depicted in Figure 1. The overall initial contamination with *C. albicans* was relatively high ($1.85 \times 10^6 \pm 2.7 \times 10^6$ CFU mL^{-1}), and no significant differences in mean counts were detected among groups at T1 ($p > 0.05$, Kruskal-Wallis test). Some variation in contamination among different teeth could be observed within each group, as represented by the outliers. Over time, significant reductions ($p < 0.05$, Friedman test) in mean counts of *C. albicans* were detected within all groups (Figure 2), particularly from pre-treatment (T1) to CMD (T2), and from pre-treatment (T1) to post-intracanal medication (T3) ($p < 0.05$, Wilcoxon test). An additional significant reduction in *C. albicans* levels from T2 and T3 was observed only in the Endovac group ($p = 0.03$, Wilcoxon test). When the mean reduction in microbial counts from T1 to T3 was evaluated (Figure 3), the Endovac group presented the highest value ($2.2 \times 10^6 \pm 0.8 \times 10^5$ CFU mL^{-1}) and the PDT had the lowest mean reduction ($1.4 \times 10^6 \pm 0.5 \times 10^5$ CFU mL^{-1}). Nevertheless, no significant differences were observed among groups ($p > 0.05$, Kruskal-Wallis test). By contrast, the mean levels of *C. albicans* at the final evaluation (T3) were significantly lower in the Endovac group compared to the PDT group (Figure 2; $p > 0.05$, Mann-Whitney test). No other differences in microbial mean counts were detected among groups at T2 or at T3 ($p > 0.05$, Kruskal-Wallis test).



The horizontal lines represent the median of CFU/mL^{-1} of *C. albicans* in each group, and the whiskers indicate the minimum and maximum values.
PDT: photodynamic therapy. No significant differences were observed among groups (Kruskal-Wallis test; $p > 0.05$).
°* refer to outliers.

Figure 1. Distribution of pre-therapy (T1) levels of *C. albicans* (CFU/mL-1) in contaminated teeth of four therapeutic groups.

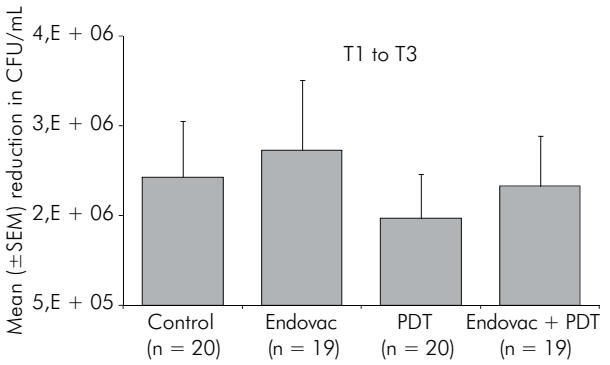


T1: pre-therapy sampling; T2: sampling after the therapeutic protocols; T3: sampling after intracanal medication. PDT: photodynamic therapy.
†Refers to significant differences within each group over time ($p < 0.05$, Friedman test).
§Refers to significant difference from T2 to T3 in the Endovac group ($p < 0.05$, Wilcoxon test).
*Refers to significant difference in mean counts between the Endovac and PDT groups at T3 ($p < 0.05$, Mann-Whitney test).

Figure 2. Changes in mean (±SEM) levels of *C. albicans* (CFU/mL^{-1}) over time in the four therapeutic groups.

Discussion

This study evaluated the *ex vivo* antimicrobial effect of the EndoVac system and PDT associated with CMD plus intracanal $Ca(OH)_2$ against *C. albicans* biofilm in the root canal system. This opportunistic pathogen is the main yeast associated with persistent endodontic infections, suggesting *C. albicans* may play an important role in endodontic clinical practice. Presumably, its persistence in the root canal may be due to its capability



No significant differences were observed among groups (Kruskal-Wallis test; $p > 0.05$).

Figure 3. Bar chart of mean (±SEM) reduction in levels of *C. albicans* (CFU/mL-1) from pre-therapy (T1) to post-therapy, after intracanal medication (T3).

to survive in a hostile environment, such as in the root canal system after treatment and biofilm formation.[4,6,7,8] A prolonged time of contamination with *C. albicans* was used in order to increase intracanal biofilm formation and penetration into dentinal tubules in the form of hyphae and yeast.[5,15] In fact, relatively high mean levels of pre-therapy contamination could be detected in most teeth in all groups, even though some variability was observed. This is expected because of anatomical variations and the complexity of the premolar root canal system.[17,18]

Regarding the antimicrobial efficacy of the four therapeutic protocols tested against *C. albicans*, all treatments were able to significantly reduce the numbers of this microorganism. Most of this reduction took place right after CMD, which indicates that conventional endodontic therapy is efficient enough to achieve high rates of microbial disinfection. An additional significant decrease after intracanal medication occurred only in the Endovac group, reinforcing the beneficial role of intracanal dressing as an adjunctive antimicrobial agent in reducing or eliminating residual microorganisms after CMD.[1,19] The current data indicate that the EndoVac system yielded the lowest mean counts of *C. albicans* at the final evaluation (T3). By contrast, the PDT group presented the lowest efficacy. Of interest, the combination of the EndoVac system with PDT had an antimicrobial effect similar to that of the control. Based on these findings, one could say that the EndoVac system improved the efficacy of PDT when combined with this therapeutic protocol.

Several studies have shown the higher efficacy of the EndoVac system in removing debris and the smear layer, as well as in improving the antimicrobial effect of irrigants, especially in the apical area, when compared with other irrigation systems.[10,20,21] Conversely, other *in vivo* and *ex vivo* investigations did not demonstrate greater advantages of the EndoVac system over other irrigation systems.[9,15,16,22] Conflicting data on the antimicrobial efficacy of the EndoVac system may be explained by critical methodological differences amongst studies, such as the volume, concentration and type of the irrigant solution.[10,20,21] In the present study, care was taken regarding the use of a standard irrigant (NaOCl 5.25%) and of the same volume (±21 mL) for all groups. Also, the selection of the target microorganism may partially account for these distinct results. No studies so far have evaluated the effect of the EndoVac system on an *ex vivo* or *in vitro* model of endodontic infection with *C. albicans*. Notwithstanding, the EndoVac system has the greatest advantage of delivering the irrigating solution to the WL with minimal risk of periapical extrusion, representing a safe method of irrigation/aspiration.

The application of PDT as an adjunctive antimicrobial procedure to CMD in root canal treatment is still under investigation. Some studies indicate additional reduction in microbial numbers when PDT is used in combination with conventional endodontic treatment.[13,14] Ng *et al.* [13] evaluated the antimicrobial effects of PDT on freshly extracted teeth with pulpal necrosis and showed that PDT significantly reduced residual bacteria within the root canal system after CMD.[13] In relation to *C. albicans*, only two studies evaluated the antimicrobial effect of PDT on this species. Those authors demonstrated that this microorganism was susceptible to lethal photosensitization using low-power laser irradiation.[23,24] However, PDT was tested directly on pure culture of planktonic *C. albicans* or its biofilm, limiting the comparisons between our data and those studies.[13] As a matter of fact, discrepancies in the *in vitro* and *in vivo* effectiveness of new adjunctive alternatives to conventional endodontic therapy, such as PDT, hinder data interpretation. A recent systematic review by Fransson *et al* [25] evaluated the *in vivo* efficacy of PDT as an adjunct to chemomechanical disinfection of infected root canals.[25]

The authors were unable to perform a meta-analysis due to the low quality and high heterogeneity of the investigations regarding study design, treatment protocols, and outcome measurements. They pointed out the need for the development of standardized therapeutic protocols in further clinical studies, considering a defined low-power laser irradiation, its total power and wavelength, time of irradiation, as well as the specificity, concentration and time of pre-irradiation of the photosensitizer. In the current investigation, for instance, methylene blue was used as a photosensitizer due to its proven effectiveness.[26]

Conclusion

Based on the data from this *ex vivo* study, the EndoVac irrigation/aspiration system combined with conventional CMD and intracanal calcium hydroxide medication presented the highest antimicrobial efficacy against intracanal *C. albicans*. PDT did not present any additional antimicrobial benefit compared to CMD in the treatment of endodontic *C. albicans* infection. However, these findings must be interpreted carefully due to the limitations of the *ex vivo* experimental models compared to *in vivo* studies of therapeutic efficacy.

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