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Effect of Apical Size and Taper on the Efficacy of Root Canal Disinfection With LED Photodynamic Therapy as an Adjunct to Irrigation With Sodium Hypochlorite

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Abstract

Introduction: This study assessed the effect of apical size and taper on the efficacy of root canal disinfection with LED photodynamic therapy (PDT) as an adjunct to irrigation with sodium hypochlorite.

Methods: A total of 126 extracted human mandibular molars were divided into 4 groups. The mesiobuccal canal was prepared to size 25/4% in group 1, 25/6% in group 2, 30/4% in group 3, and 30/6% in group 4 using the iRaCe rotary system. A 21-day *Enterococcus faecalis* biofilm was prepared and used for inoculation of the canals. Each group was randomly divided into 3 subgroups for canal disinfection with 2.5% sodium hypochlorite, sodium hypochlorite plus LED PDT and saline (positive control). Samples from the root canals were obtained with rotary files and cultured. Microbiologic data were analyzed using the Poisson regression test.

Results: The bacterial count significantly decreased following disinfection with sodium hypochlorite with/without PDT in all sizes and tapers of preparation compared with the control group ($P<0.05$). Increasing the apical taper or apical size and the use of PDT as an adjunct did not have a significant effect on the reduction of the bacterial count ($P>0.05$). However, the apical size and PDT had a significant effect on the number of residual bacteria ($P<0.05$), and increasing the apical size and conduction of PDT significantly decreased the number of residual bacteria.

Conclusion: The apical size and taper and the use of PDT as an adjunct did not have a significant effect on the reduction of the bacterial count. However, increasing the apical size and conduction of PDT as an adjunct to sodium hypochlorite irrigation significantly decreased the number of residual bacteria in the root canal system.

Keywords: *Enterococcus faecalis*; Sodium hypochlorite; Photodynamic therapy; Apical size; Taper.

Introduction

Bacteria and their products are the main cause of endodontic diseases. Thus, elimination or reduction of bacterial contamination is the main goal of endodontic treatment. This goal can be achieved by proper chemomechanical preparation of the root canal system while conserving the maximum tooth structure and the original canal shape. However, some residual bacteria may remain in the root canal system and penetrate deep into dentinal tubules, accessory canals and their branches, apical delta or transverse anastomoses.[1] Due to the complex nature of the root canal system, cleaning and shaping alone may not efficiently decrease the bacterial count.[2]

Microorganisms remaining in the apical portion of the root canal system are mainly responsible for endodontic treatment failure. Primary endodontic infections often have a poly-microbial nature, and anaerobic gram-negative rods are the dominant culprits responsible for their occurrence. In contrast, only a few bacterial species are involved in secondary endodontic infections.[3]

Enterococcus faecalis is a resistant microorganism that comprises a small portion of the microbial flora of an untreated root canal system. However, it plays an important role in the development of refractory peri-radicular lesions after root canal treatment. *E. faecalis* is

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commonly isolated from a high number of cases of root canal treatment failures and can survive in the root canal as a single microorganism or as part of the microbial flora.[3]

Root canal preparation with larger instruments enables the access of a larger volume of irrigant to the apical region.[4] On the other hand, over-preparation results in greater dentin removal and a higher risk of procedural errors such as root canal zipping, canal transportation, root perforation and root fracture.[5]

Sodium hypochlorite is the most widely used endodontic irrigating solution, which is used alone or in combination with a chelator to decrease the bacterial count or eliminate the smear layer. However, controversy exists regarding the most appropriate concentration of this antibacterial agent and its safety and biocompatibility.[6]

Photodynamic therapy (PDT) is a new antimicrobial method, which involves the application of a non-toxic photosensitizer and a light source (laser or LED lamp) and has high potential for the reduction of the bacterial load. The risk of thermal damage in PDT with an LED is very small compared with the use of high-power lasers, and it is much more cost-effective. Moreover, evidence shows its higher efficacy than lasers for disinfection. PDT has antibacterial effects against *E. faecalis*, which is the main culprit responsible for many cases of endodontic treatment failure.[7]

A search of the literature by the authors yielded no previous study on the effects of apical size and taper on the efficacy of root canal disinfection with LED PDT as an adjunct to sodium hypochlorite irrigation. Thus, this study aimed to evaluate the effect of apical size and taper on the efficacy of root canal disinfection with LED PDT as an adjunct to irrigation with sodium hypochlorite.

Materials and Methods

This in vitro experimental study was conducted on the mesiobuccal canals of extracted human mandibular first and second molars. The teeth had been extracted due to periodontal disease, orthodontic treatment or severe caries.

The factors under study were apical size and taper at 4 levels (Table 1) and 2 methods of root canal disinfection: (1) NaOCl irrigation, (2) NaOCL irrigation + PDT. The sample size was calculated to be 10 in each subgroup (a total of 80) according to a previous study by Tabachnick and Fidell,[8] assuming 90% study power, alpha = 0.05 and beta = 0.2. In order to have a positive and a negative control group and also assess the biofilm formation under an electron microscope, a total of 126 samples were collected.

The inclusion criteria were extracted human mandibular first and second molars with completely formed roots. The exclusion criteria were root caries, root calcification, previous endodontic treatment, cracks, resorption, root curvature >20°, presence of lateral foramen or apical constriction larger than size 15. Root curvatures were measured using the method proposed by Pruett et al.[9] After access cavity preparation, the presence of two separate mesial canals was confirmed by inserting #10 K-files into the canals and observing the tips at the apical foramina. The teeth were included using convenience sampling.

A total of 126 teeth were collected and stored in normal saline. Hard and soft tissue residues were removed and the teeth were immersed in 5.25% sodium hypochlorite for 24 hours. They were then transferred into sterile saline (0.9%) at room temperature until the experiment.

The teeth were decoronated at the cementoenamel junction perpendicular to the longitudinal axis of the tooth using a high-speed disc under copious water irrigation such that the remaining root had a 10-11 mm length. After access cavity preparation, the presence of two separate mesial canals was ensured by placing #10 K-files into the mesial root canals such that the tip of the files was visible at the apical foramina.

For the purpose of standardization, the root canals of all teeth underwent similar chemomechanical

Table 1. Preparation of Root Canals in the Four Groups

Group	Order of File Size	Order of File Taper
1	20	2%
	25	2%
	25	4%
2	20	2%
	25	2%
	25	4%
	25	6%
3	20	2%
	25	2%
	25	4%
	30	4%
4	20	2%
	25	2%
	25	4%
	30	4%
	30	6%

Table 2. Colony Count in the Groups

Apical Size and Taper	Disinfection Method		
	Positive control	Sodium Hypochlorite	Sodium Hypochlorite + PDT
25/4%	1.97×10^{15}	3.09×10^{13}	1.07×10^{13}
25/6%	4.01×10^{15}	3.69×10^{13}	1.63×10^{13}
30/4%	1.57×10^{15}	1.18×10^{13}	6.3×10^{12}
30/6%	7.32×10^{15}	4.1×10^{13}	1.1×10^{12}

preparation. The teeth were randomly divided into four groups (n = 30) using a table of random numbers. Each group was instrumented using iRaCe (FKG, Dentaire, La-Chaux-de-Fonds, Switzerland) rotary files with an endo-motor (X-SMART, Dentsply, Ballaigues, Switzerland) according to the manufacturer's instructions. Coronal flaring was performed using file size 35/8%. A #10 K-file (Dentsply Maillefer, Ballaigues, Switzerland) was inserted into the root canal until its tip was visible at the apex; the working length was calculated by subtracting 1 mm from this length. The canals were then prepared with iRaCe rotary files operating at 600 rpm and 2 N/cm torque. All rotary files were inserted into the canals up to the working length (Table 1). During the preparation process, the root canals were rinsed with 10 mL saline between using each of the two files. After using each file, patency was ensured using the #10 K-file to 1 mm beyond the working length. In case of file fracture, the tooth would be excluded and replaced. For smear layer removal, the root canals were rinsed with 1 mL of 17% EDTA for 3 minutes followed by a final rinse with 5 mL of 5% sodium hypochlorite and 5 mL of saline. The apical foramen was then sealed with a glass ionomer (GC fuji II, Tokyo, Japan)[10] to avoid apical extrusion during irrigation.[11]

The teeth underwent autoclave sterilization at 121°C and 15 psi pressure for 15 minutes. Each tooth was placed in a test tube containing 1 mL of brain heart infusion (BHI) broth (Merck, Germany). The tubes were then capped and autoclaved. To ensure the absence of microbial contamination, five teeth were selected as the negative control group.

All root canals except for those in the negative control group were then inoculated with *E. faecalis* obtained from the Pasteur Institute of Iran.

For biofilm formation in dentinal microtubules, first *E. faecalis* was cultured in BHI agar and then a bacterial suspension with 0.5 McFarland standard concentration (1×10^8 CFUs/mL) and optical density of 0.09 at a 600 nm wavelength (measured by a spectrophotometer) was prepared using saline. The teeth were immersed in BHI broth in microtubes and 50 µL of bacterial suspension was inoculated into the canal. All tubes were then incubated at 37°C for 21 days[12] to allow biofilm formation and its penetration into dentinal tubules. During this time period, the BHI broth was refreshed once every other day and the tubes were vortexed to allow the complete penetration of the culture medium and bacteria into dentinal tubules. Also, in order to ensure bacterial proliferation and its purity, the samples were randomly taken from some teeth in the experimental and negative control groups and passaged on the agar culture medium. They were then incubated for 24 hours. The homogeneity of colonies and the absence of contamination were ensured as such.[13] This subculture was repeated several times during the 21-day incubation period.

For the intervention, first the culture medium was extracted and the root canals were gently rinsed with saline and dried with sterile paper points. Next, they were placed on a sterile plate. To prevent bacterial contamination of root canals, the surface of the teeth was coated with nail varnish. All phases of intervention were performed under a hood. The teeth in each group were then randomly divided into three subgroups.

Subgroup 1. Sodium hypochlorite: The root canals were irrigated with 1 mL of 2.5% sodium hypochlorite using an insulin syringe to the apical 1 mm. It remained in the canal for 60 seconds.[14] Next, the root canals were rinsed with sodium thiosulfate for 30 seconds and finally with 5 mL of saline. Excess moisture in the pulp chamber was removed using a sterile cotton pellet. For sample collection, a file one size larger (both apical size and taper) than the master apical file of the RaCe rotary system (size 30/6% for the 25/4% group, size 30/8% for the #25/6% group, size 35/6% for the 30/4% group and size 35/8% for the 30/6% group) with the X-Smart rotary motor was introduced into the canal to the working length and remained there for 30 seconds, operating at 600 rpm and 2 N.cm torque. Next, the file was separated and placed in a microtube containing BHI broth with a sterile hemostat. This was done to allow the proliferation of bacteria in dentinal debris. All the procedures were performed under a class II sterile hood.

Subgroup 2. Sodium hypochlorite + PDT: In this group, sodium hypochlorite was first used as in group 1. The root canals were dried with paper points and then 0.1 mg/mL toluidine blue photosensitizer was injected into the canal using a 30-gauge needle and remained there for 60 seconds according to the manufacturer's instructions. The root canals were then subjected to LED (Fotosan; CMS Dental, Copenhagen, Denmark) irradiation for 60 seconds with a 600 nm wavelength and 200 mW/cm^2 power. They were then rinsed with 5 mL of saline, and the samples were collected from the root canals as in subgroup 1.

Subgroup 3. Positive control: The positive control teeth were of the same apical size and taper as those of the experimental groups. For example, for the 20 teeth prepared to size 25/4%, 10 positive control teeth prepared to size 25/4% were used. The teeth in this group were rinsed with 10 mL saline with a 30-gauge needle. No other intervention was performed. The samples were obtained as in subgroup 1.

In order to ensure biofilm formation, one sample was used for evaluation under an electron microscope. It was prepared as in the experimental groups, and longitudinal grooves were carved on its buccal and lingual surfaces. Similar to other teeth, this sample underwent *E. faecalis* culture for 21 days. The tooth was then split into halves using a sterile chisel and hammer at the site of the grooves. The samples were then immersed in 2.5% glutaraldehyde

at 4°C for 24 hours to fix the bacteria and evaluated under a scanning electron microscope (Leo Electron Microscopy Ltd, Cambridge, UK) (Figure 1).

Samples were taken from the root canals using files. The files were then transferred to sterile microtubes containing BHI broth. They were then incubated at 37°C for 16 hours. In order to easily count the colonies on a plate, the proliferated bacteria were diluted in microtubes by 1:10. In order to dilute the samples after vortexing and homogenizing the infected dentin particles, 40 µL of the suspension was collected and added to 460 µL of sterile saline in a 96-well plate (dilution by 1:10). Next, 500 µL of the first dilute was added to the second well and serial dilution was continued as such. All groups were diluted as 1:10, 1:100, and so on to $1:10^{10}$. Next, 100 µL of the diluted solution was spread-cultured on 8x8 cm plates containing BHI agar and incubated for 24 hours. The colony count of *E. faecalis* was then determined using a colony counter and reported as colony-forming units per milliliter (CFUs/mL).

In order to compare the experimental groups in terms of reduction in the colony count, the percentage reduction of the colony count was calculated.

The number of colonies in the positive control group (no treatment) was used as the baseline value for the purpose of comparison with the colony count in the experimental groups.

The Poisson regression was applied to assess the effect of apical size, apical taper and method of root canal disinfection on the colony count. The data were analyzed using STATA 12 software. $P \leq 0.05$ was considered statistically significant.

Results

Table 2 shows the colony count in the groups. The mean percentage reduction of colony counts in the experimental groups compared with the positive control group was 98.48% in sodium hypochlorite and 99.46% in sodium hypochlorite plus PDT in the 25/4% group. These values were 98.25% and 99.59% in the 25/6% group and 99.44% and 99.98% in the 30/6% group, respectively. The decrease in the colony count was significant in all experimental groups compared with the positive control group ($P < 0.05$).

The assessment of data distribution in all groups showed that the distribution of the data was close to Poisson distribution. Thus, Poisson regression and then the goodness of fit analysis were performed. The goodness of fit analysis showed a good fit of the data with Poisson regression:

Deviance goodness-of-fit = 0.805514
Prob > chi2(76) = 1.0000
Pearson goodness-of-fit = 0.8018983
Prob > chi2(76) = 1.0000

Thus, Poisson regression was used to compare the groups in terms of the percentage of reduction in the bacterial count and also to assess the effect of apical size, apical taper and method of disinfection on the colony count. The results showed that although increasing the apical size from 25 to 30 caused a greater reduction in the bacterial count in the root canal system, this reduction was not statistically significant ($P = 0.794$). Increasing the apical taper from 4% to 6% did not increase the reduction in the bacterial count either ($P = 0.947$). Although the use of PDT as an adjunct to sodium hypochlorite resulted in a higher reduction in the bacterial count compared with the application of sodium hypochlorite alone, the difference was not statistically significant ($P = 0.702$).

The adjustment analysis of all three factors of size, taper and disinfection technique was then performed to assess the effect of each variable on the percentage of reduction at the same time. The results confirmed the previous findings regarding no significant effect of these factors on bacterial reduction ($P > 0.05$).

Next, we used Poisson regression to analyze the percentage of residual bacteria in the root canal. The results showed that when the dependent variable was changed from the percentage of reduction to the percentage of residual bacteria in the root canal, the effects of apical size ($P = 0.003$) and the disinfection

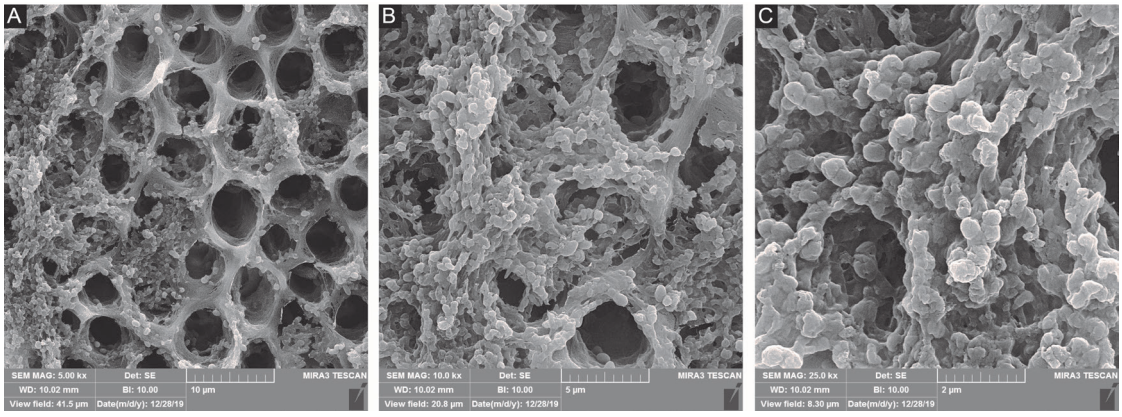

Figure 1. SEM Images of the Root Canal Wall After 3 Weeks of Incubation with *Enterococcus faecalis*: A (×5000), B (×10000), C (×25000).

method ($P=0.000$) were significant on the percentage of residual bacteria. However, the effect of apical taper was not significant ($P=0.449$). The Poisson's regression was then performed to compare the antibacterial effects of sodium hypochlorite alone and in combination with PDT for both apical sizes 25 and 30. The results showed that the application of PDT as an adjunct to sodium hypochlorite caused a significant reduction in the count of residual bacteria in both apical sizes 25 ($P=0.001$) and 30 ($P=0.022$).

Thus, preparation to apical size 30 (compared with 25) and the use of PDT as an adjunct to sodium hypochlorite irrigation caused a significant reduction in the residual bacterial count in the root canals. However, increasing the apical taper had no significant effect on the count of residual bacteria.

Discussion

To the best of the authors' knowledge, this study was the first to assess the effect of apical size and taper on the efficacy of root canal disinfection with LED PDT as an adjunct to sodium hypochlorite irrigation. The results showed that the bacterial count significantly decreased following disinfection with sodium hypochlorite with/without PDT in all sizes and tapers of preparation compared with the control group ($P<0.05$). Increasing the apical taper or apical size and the use of PDT as an adjunct did not have a significant effect on the reduction of the bacterial count ($P>0.05$). However, apical size and PDT had a significant effect on the number of residual bacteria ($P<0.05$), and increasing the apical size and PDT significantly decreased the number of residual bacteria.

Sodium hypochlorite has unique properties such as the ability to change or inhibit cell metabolism, high antibacterial activity and an alkaline pH. It is the most commonly used endodontic irrigating solution.[15,16] Thus, sodium hypochlorite was used for root canal disinfection in this study.

Akhlaghi et al[4] reported that apical preparation of mesiobuccal canals to size 25/4% had no significant difference with other sizes (35/6%, 35/4%, 30/6%, 30/4% and 25/6%) and the bacterial count equally and efficiently decreased in all groups. Their results were similar to our findings. Arvaniti and Khabbaz[17] evaluated the effect of apical taper on cleaning efficiency of root canals and showed that apical size 30 with 4%, 6% and 8% tapers had no significant difference in the rate of debridement. Aydin et al[18] also compared the effects of conservative and invasive filing on the number of residual bacteria and showed that invasive dentin removal was not significantly different from conservative cleaning regarding the reduction in the bacterial count.

Srirekha et al[19] reported that increasing the taper from 4% to 6% in apical size 30 resulted in more efficient and deeper penetration of irrigating solution into the apical region. Controversy in the results may be due to different assessment techniques. Srirekha et al[19] used radiopaque dye for the assessment of penetration and efficacy of disinfecting solution while we performed microbial culture to assess the reduction in the bacterial count.

Paraskevopoulou and Khabbaz[20] evaluated the effect of taper on the reduction of the bacterial count and found that changing the size from 20 to 30 and changing the taper from 4% to 8% caused a significant difference in the number of residual bacteria. The difference in the results of the two studies may be due to the fact that they assessed planktonic bacteria while we evaluated bacterial biofilm. The technique of using the file in the canal and the assessment of different types of teeth can also affect the results.

Our results indicated that the conduction of PDT as an adjunct to root canal irrigation with sodium hypochlorite could not significantly contribute to a bacterial reduction in the root canals. Souza et al[21] evaluated the efficacy of PDT with two different photosensitizers for the enhancement of root canal disinfection and removal of *E. faecalis*. They found that irrespective of the type of photosensitizer used, PDT had no significant effect on the reduction of the bacterial count following routine chemomechanical preparation. Similarly, da Rosa et al[12] assessed the effect of PDT on the bacterial count in the root canal system and found no significant difference in comparison with sodium hypochlorite and chlorhexidine. Samiei et al[14] and Janani et al[22] investigated the effectiveness of PDT for root canal disinfection in comparison with sodium hypochlorite and showed that despite the significant efficacy of PDT compared with the control group, sodium hypochlorite was the most effective method for decreasing the bacterial count in the root canal system. Asnaashari et al[23] used PDT with an LED at a 630 nm wavelength and concluded that this method was significantly more effective than an 810 nm diode laser for the elimination of *E. faecalis*. However, they did not make a comparison with sodium hypochlorite.

Some studies regarding PDT reported results in contrast to ours. Tennert et al[24] and Vaziri et al[25] evaluated the antimicrobial effects of PDT and concluded that this technique as an adjunct to root canal disinfection with sodium hypochlorite caused a significant reduction in the bacterial count, which was different from our findings. They used paper points to collect samples and evaluated the planktonic form of bacteria while we collected the samples using endodontic files and used bacteria in the biofilm form. The difference in the results of the two studies can be due to the abovementioned factors. Rios et al[26] performed PDT after rinsing the canal with sodium hypochlorite and reported that it caused a significantly higher reduction in the bacterial count compared with the use of sodium hypochlorite alone. This result was different from our finding. The difference between our

results and theirs may be due to the use of different rotary systems and duration of bacterial inoculation, which was 14 days in their study versus 21 days in our study.

Several factors affect the efficacy of PDT in eliminating the bacteria from the root canal system, including the type of photosensitizer, time duration and use of a laser instead of an LED.[27,28] Toluidine blue and methylene blue are among the most commonly used photosensitizers in endodontic treatment. Evidence shows that toluidine blue has antibacterial effects on oral bacteria even in the absence of light radiation.[29,30] Zeina et al[31] reported that toluidine blue had maximum absorption and maximum photodynamic activity at a 630 nm wavelength. Nielsen et al[32] showed that the use of 0.1 mg/mL toluidine blue in PDT with an LED resulted in the complete elimination of *E. faecalis* after 30 seconds. Chiniforush et al[28] in a review study concluded that the use of 0.1 mg/mL toluidine blue or methylene blue for 1 to 5 minutes is the best protocol for PDT. We also used toluidine blue in this study; however, since we evaluated the bacterial biofilm, we increased the time of light radiation to 1 minute. Yildirim et al[33] reported that 1-minute light radiation is adequate to achieve maximum antimicrobial efficacy against *E. faecalis*.

In our study, similar to some previous studies, sodium thiosulfate was used to neutralize the effects of sodium hypochlorite remaining in the canal.[34,35] This was done to eliminate the antibacterial effects of sodium hypochlorite remaining in dentinal tubules, which could serve as a confounding factor. However, it is less commonly used in the clinical setting and has been disregarded in some studies.[36]

It should be mentioned that in most studies on the antimicrobial efficacy of materials/techniques, some residual bacteria may remain in hard-to-access areas such as isthmuses or ramifications. Thus, an accurate assessment of the number of residual bacteria is not possible. Instead, reduction in the bacterial count in the root canal system is often measured as an alternative to the count of residual bacteria.[37] Similarly, we first measured the reduction in the bacterial count in the root canal system. However, since the number and type of microorganisms remaining in the root canal system can determine the long-term success/failure of endodontic treatments, the number and type of residual bacteria in the root canal are of clinical significance. For this reason, we also analyzed the number of residual bacteria in the root canal system, which revealed that larger apical size and application of PDT in addition to the use of sodium hypochlorite caused a significantly higher reduction in the number of residual bacteria. The difference between the results of statistical analyses of bacterial count reduction and reduction in the number of residual bacteria is due to the smaller values and their higher sensitivity to statistical analyses, causing a significant difference among the groups in the apical size and type of disinfection. However, the effect of apical taper remained insignificant.

This study had an in vitro design and since the results of statistical analyses of the reduction in the bacterial count and residual bacteria were different, future clinical studies with a large sample size are required to assess the effect of apical size and taper and method of disinfection on clinical signs and symptoms of patients. Moreover, since no standard protocol is available for PDT and such factors as the type of photosensitizer, light source and radiation time can affect the results, further studies are required to suggest a standard protocol for PDT. Furthermore, the effect of novel techniques such as ultrasonic activation of sodium hypochlorite on the efficacy of root canal disinfection in different apical sizes and tapers must be investigated in future studies. Last but not least, considering the significance of the number of residual bacteria in the treatment outcome, methods should be suggested to more accurately determine the count of residual bacteria.

Conclusion

Within the limitations of this study, the results showed that increasing the apical size and use of PDT as an adjunct to root canal disinfection with sodium hypochlorite can significantly decrease the number of residual *E. faecalis* in the root canal system. This finding may be of clinical significance.

Ethical Considerations

This study was approved by the ethics committee of Shahid Beheshti University of Medical Sciences (IR.SBMU.DRC.REC.1397.065).

Conflict of Interest

The authors declare no conflict of interest.

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