The Role of Apical Size Determination and Enlargement in the Reduction of Intracanal Bacteria

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Abstract

The master apical preparation size in root canal therapy is debatable despite considerable research. The present study compared file sizes that bind at the apex before and during crown-down preparation and assessed the relation between apical size and extent of intracanal bacterial load. There were 100 single-rooted teeth biomechanically prepared after inoculation with *Enterococcus faecalis*. Canals were preflared, and apical size was ascertained by the first file to bind (FAB) at the working length (WL). During crown-down preparation, the first crown-down file to reach the apex during instrumentation was noted (CDF). Teeth were then divided into three master apical file size groups of CDF + 1, CDF + 2, and CDF + 3. Positive controls were inoculated postinstrumentation, whereas negative controls were instrumented without inoculation ($n = 5$). The samples were then cultured for intracanal bacterial counts. Fifteen samples and four controls were analyzed under SEM. The data were analyzed using ANOVA, Student's *t*-test, and χ^2 tests. The CDF was demonstrated to be an average of four file sizes larger than the FAB ($p < 0.05$). There was a significant increase in the number of samples with negative cultures from CDF + 1 to CDF + 3. SEM observation revealed bacteria on dentinal walls and in tubules even in most negative canal cultures. *(J Endod 2006;xx:xxx)*

Key Words

Apex size, apical binding, apical enlargement, apical size, bacteria, canal culturing, crown down, dentinal tubules, *Enterococcus faecalis*, intracanal, master apical file, SEM, scanning electron microscopy

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The aim of root canal treatment is to eliminate bacteria from within the canal system to create an environment favorable for healing. Current preparation techniques along with disinfectants or medicaments may disrupt the intracanal microbial environment. However, numerous studies have shown that it is impossible to achieve a bacteria-free root canal consistently (1–4). Hence, there is concern over the consequences of the presence of the remaining microorganisms in the canal. It is generally believed that the remaining bacteria can be either eradicated or prevented from recolonizing the root canal system through an interappointment medicament such as calcium hydroxide (2, 5). However, it has been demonstrated that calcium hydroxide consistently fails to sterilize root canals and may even allow regrowth in some cases (3, 4, 6). The presence of cultivable microorganisms at the time of obturation has been reported to impair healing after root canal therapy (7).

During canal preparation, apical size has been crucial in defining successful debridement of the root canal system. Recently, Khademi et al. (8) analyzed debris from the apical third of root canals under SEM. It was concluded that the minimum instrumentation size needed for penetration of irrigants to the apical third of the root canal was a #30 file. This has confirmed earlier findings that larger sizes are needed for irrigating closer to the apex (9–12). Larger apical size preparations have also demonstrated greater microbial reduction in the apical third (3, 13–18). However, no technique that completely cleans the apical canal space has been demonstrated (19–21).

The master apical file (MAF) size has been related to the initial apical size in many studies. Historically, the "three sizes up from the first file to bind" rule was and is still being used in modified forms (22). More recent studies report that initial flaring before determining the apical size may give a more accurate measurement of the apex (19, 23). Tan and Messer (24) reported that the apical diameter proved to be at least one file size bigger once preflaring was done. Contreras et al. (23) reported the apical size to be two file sizes bigger after preflaring with Gates-Glidden drills. In addition, Pecora et al. (25) reported that the instrument used for preflaring played a major role in determining the anatomical diameter at the working length (WL). Hence, the anecdotal rule of "three files up" may not be useful without a proven method to judge apical size before instrumentation, and three has yet to be confirmed as the magical number to apical cleanliness. In principle, however, preparing each canal to a specific apical diameter as per its initial apical size may better equip the clinician to provide a more predictable canal preparation.

The objectives of this study were to evaluate methods of determining initial apical size and secondly to assess the MAF size according to the initial apical size and its effect on intracanal bacterial load.

Materials and Methods

There were 100 single-rooted teeth decoronated at 14 mm from the apex. Working lengths were set at 1.0 mm from the foramen under the microscope. Samples were also radiographed to ensure proper measurement. A turbidity determination chamber was made by inserting penrose tubing over the coronal end and mounting each sample in a glass vial through a perforated lid. Cyanoacrylate resin was used to seal both ends, and nail polish was applied around the radicular surface to prevent lateral leakage. Blood heart infusion (BHI) broth was pipetted into the vials to submerge the apical 2 mm of each sample. An inoculum of *Enterococcus faecalis* was placed in contact with the coronal surface of each sample daily until turbidity of the broth was observed, indicating

bacterial contamination of the root. The contaminated samples were allowed to air-dry for 10 minutes before biomechanical preparation.

Coronal preflaring was performed on all canals using 02 K-files #10 to #30 and Gates Glidden drills #2 through #4 within the coronal two-thirds of the canal. At this point, sequentially larger K-files were inserted to WL until the first file to bind at the WL (FAB), which was recorded. The apical one-third of each canal was then instrumented in a crown-down fashion using Profile .04 files and irrigating with 5.25% sodium hypochlorite. The first file to reach the WL was recorded as the crown-down file (CDF). The samples were randomly separated into three groups of 30 samples each and control groups of 5 each. In group CDF + 1, the MAF was determined as one file size larger than the determined CDF, while in groups CDF + 2 and CDF + 3, the MAF size was 2 and 3 file sizes larger, respectively. The samples were finally irrigated with 10 ml of sodium hypochlorite, and subsequently with sodium thiosulfate to neutralize the sodium hypochlorite. In the control group, negative controls were not inoculated with bacteria, whereas positive controls were inoculated after cleaning and shaping the canals.

After drying the canals using sterile paper points, each canal was filled with phosphate-buffered solution (PBS) and agitated lightly with a sterile file smaller than MAF. PBS was extracted from the canal using sterile paper points and placed into a 1-ml vial of PBS. The vial was vortexed to obtain a suspension of bacteria that was centrifuged, resulting in a bacterial pellet. Pellets were diluted in 100 μl of PBS and plated on BHI agar. Bacterial counts were tabulated as colony forming units (CFUs) at 48 hours. Colonies were gram stained for confirmation of *E. faecalis*. Absence of bacterial colonies was considered to indicate a negative culture.

Five samples from each study group and two from each control were prepared for SEM. Teeth were longitudinally grooved and carefully split. Samples were rinsed in PBS, soaked in glutaraldehyde for 1 hour, and postfixed in 1% OsO_4 for 30 minutes. They were then dehydrated, and mounted on SEM discs, and spatter coated with palladium gold.

A Student *t* test for independent samples was performed for differences related to FAB and CDF. The CDF groups were compared using the ANOVA test for intracanal bacterial load. When differences between groups were found, the Student's *t* test for unequal variances was used to determine the level of significance. The three CDF groups were also compared using the χ^2 test for the number of negative bacterial cultures per group. Lastly, the MAF of every sample was compared for intracanal bacterial load using the ANOVA analysis. For all tests, values of $p < 0.05$ were considered statistically significant.

Results

The FAB method consistently showed smaller file sizes compared with the CDF method for all samples. The average file size using the FAB method was approximately ISO 25, whereas that for the CDF method was approximately ISO 45. The CDF was at least one ISO file size larger than the FAB ($n = 2$), with a maximum difference of six ISO file sizes ($n = 1$). The average FAB was significantly smaller than the average CDF, with a difference of approximately four ISO file sizes.

TABLE 1. Intracanal bacterial cultures from CDF groups

Bacterial load	CDF + 1	CDF + 2	CDF + 3
Mean* (positive cultures only)	59 ± 122	30 ± 65	23 ± 37
Positive culture	21 (70%)	19 (63%)	14 (47%)
Negative culture	9 (30%)†	11 (37%)	16 (53%)†
Total	30 (100%)	30 (100%)	30 (100%)

*Average CFU count per canal.
†Significant difference, $p < 0.05$.

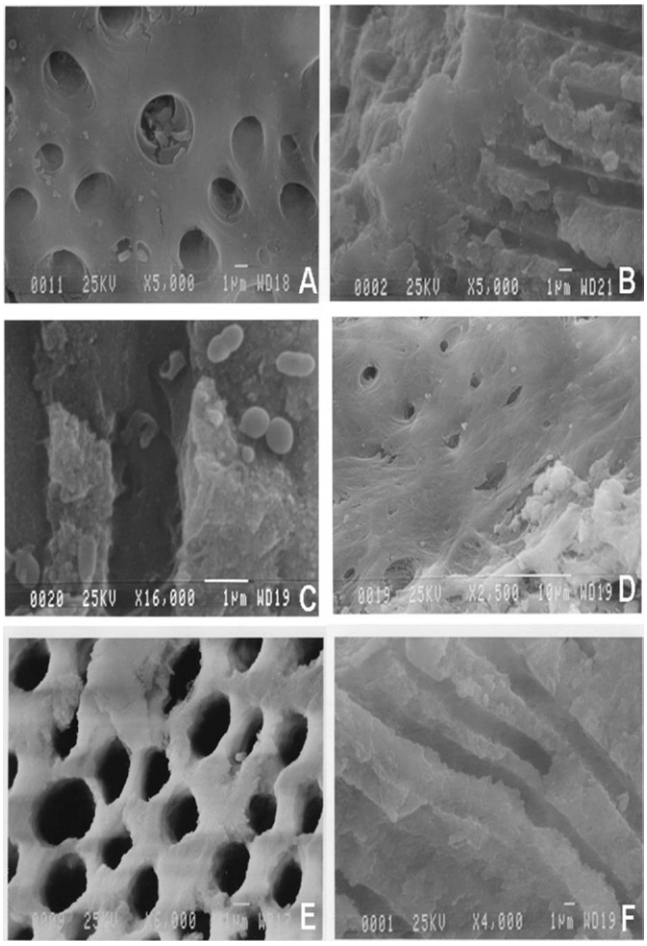

Figure 1. (*A*) Bacterial cells in a CDF + 1 sample. (*B*) Bacterial cells in a CDF + 2 sample. (*C*) *E. faecalis* cells seen within dentinal tubules. (*D*) Positive control showing dense bacterial colonies. (*E*, *F*) Negative control.

The bacterial counts were compared for MAFs ranging from ISO file sizes 50 to 60. The average bacterial counts showed no significant difference between ISO 50 and 55 and between ISO 55 and 60. Interestingly, the average bacterial counts were significantly higher in the ISO 60 MAF samples than those found in the ISO 50 MAF group.

Analysis revealed no significant differences between CDF groups, although the bacterial counts decreased between the CDF + 1 to CDF + 3 groups. Negative controls yielded no bacterial colonies, whereas positive controls yielded significantly higher bacterial counts. The groups were then compared for number of samples yielding no bacterial colonies. Table 1 reports the number of negative versus positive culture samples in each group as well as the mean number of bacterial CFUs for all samples with positive cultures. The χ^2 test revealed that the number of samples yielding a negative culture were significantly higher in the CDF + 3 group compared to the CDF + 1 group. However, no significant differences were observed between the CDF + 1 and CDF + 2 groups and between the CDF + 2 and CDF + 3 groups.

The SEM analysis revealed *E. faecalis* in all study samples observed. More bacterial colonies were observed in the CDF + 1 group (Fig. 1*A*), whereas scattered cocci were observed in the CDF + 3 group (Fig. 1*B*). Bacterial colonies were also observed within the dentinal tubules in all study groups (Fig. 1*C*). The positive control SEM sample revealed dense bacterial colonies, indicating formation of bio-films (Fig. 1*D*). No identifiable bacteria were observed in the negative control sample (Fig. 1*E*, *F*).

Discussion

In the current study, apex measurements were consistently larger with the CDF method compared to the FAB method. Compared to other studies using the FAB method, (19, 24, 25) the CDF method may provide a more accurate way of determining the apical size. Because the CDF file is placed actively to the WL and the FAB file is placed passively to the WL, it may be assumed that a one-file-size difference may not be a valid difference. However, only two samples showed a difference of one file size between the FAB and CDF methods, whereas most samples differed by four file sizes. In all samples, apical sizing and apical enlargement were greater than file size #30. This may have improved chemical debridement, resulting in effective bacterial reduction (8, 9). In contrast, Coldero et al. (26) reported no difference in intracanal bacterial reduction with chemomechanical preparation. However, minimal differences in apical instrumentation sizes between groups may have led to insignificant findings.

There were significantly higher bacterial counts in the teeth prepared to file size #60 compared to #50 MAF. This contradicts studies reporting reduction of bacterial count with increased apical enlargement (14). A comparison of the MAF sizes within CDF groups revealed that 16 of the #60 MAF teeth and only 6 of the #50 MAF teeth were CDF + 1 samples. Hence, the difference in bacterial counts may be because of the unequal distribution of MAF sizes across the CDF groups.

SEM analysis in the present study revealed *E. faecalis* in all study samples observed. Of the 15 SEM samples, 7 samples were from canals that yielded negative cultures, and 8 were from canals that yielded positive cultures. Bacterial cells were observed over the canal surface or within dentinal tubules. The inability of current cleaning and shaping techniques to reach microorganisms present in lateral canals and dentinal tubules, as witnessed by the SEM observations, needs to be taken into account. Although SEM samples demonstrated a decrease in the number of bacterial colonies between the CDF + 1 and CDF + 3 groups, and culturing revealed an increase in the number of samples with negative bacterial cultures between the CDF + 1 and CDF + 3 groups, complete debridement of the root canal may be questionable.

In the present study, CDF samples demonstrated at least a 60-fold decrease in CFUs compared to the positive control samples. All bacteria-positive CDF samples revealed $<10^3$ CFUs in the canals. In vivo culturing studies have demonstrated success in canals with $<10^3$ CFUs (4, 7). Peters and Wesselink (4) reported that seven canals with positive microbial cultures were successful, of which six root canals contained $<10^2$ CFU/ml and one canal contained 2×10^3. Although promising, microbial reductions with the CDF method as seen here are preliminary and need to be assessed under clinical conditions.

Studies have advocated using larger files to clean the apex. Although instrumenting canals to larger sizes may not be prudent in every case, minimal apical preparations based on clinical opinions are far more detrimental to the success of root canal therapy (27). An appropriate apical sizing method can help the operator avoid unnecessary enlargement of the apex whereas predictably reducing intracanal debris.

References

1. Bystrom A, Sundqvist G. Bacteriologic evaluation of the efficacy of mechanical root canal instrumentation in endodontic therapy. Scand J Dent Res 1981;89:321–8.
2. Brystrom A, Claesson R, Sundqvist G. The antimicrobial effect of camphorated paramonochlorophenol, camphorated phenol and calcium hydroxide in the treatment of infected root canals. Endod Dent Traumatol 1985;1:170–5.
3. Orstavik D, Kerekes K, Molven O. Effects of extensive apical reaming and calcium hydroxide dressing on bacterial infection during treatment of apical periodontitis: a pilot study. Int Endod J 1991;24–7.
4. Peters LB, Wesselink PR. Periapical healing of endodontically treated teeth in one and two visits obturated in the presence or absence of detectable micro-organisms. Int Endod J 2002;35:660–7.
5. Chong BS, Pitt Ford TR. The role of intracanal medication in root canal treatment. Int Endod J 1992;25:97–106.
6. Reit C, Dahlen G. Decision making analysis of endodontic treatment strategies in teeth with apical periodontitis. Int Endod J 1988;21:291–9.
7. Sjogren U, Figdor D, Persson S, Sundqvist G. Influence of infection at the time of root filling on the outcome of endodontic treatment of teeth with apical periodontitis. Int Endod J 1997;30:297–306.
8. Khademi A, Yazdizadeh M, Feizianfard M. Determination of the minimum instrumentation size for penetration of irrigants to the apical third of root canal systems. J Endod 2006;32:417–20.
9. Falk KW, Sedgley CM. The influence of preparation size on the mechanical efficacy of root canal irrigation in vitro. J Endod 2005;31:742–5.
10. Chow T. Mechanical effectiveness of root canal irrigation. J Endod 1983;9:475–9.
11. Shuping G, Orstavik D, Sigurdsson A, Trope M. Reduction of intracanal bacteria using nickel-titanium rotary instrumentation and various medications. J Endod 2000;26: 751–5.
12. Siqueira J, Lima K, Magalhaes F, Lopes H, de Uzeda M. Mechanical reduction of the bacterial population in the root canal by three instrumentation techniques. J Endod 1999;25:332–5.
13. McGurkin-Smith R, Trope M, Caplan D, Sigurdsson A. Reduction of intracanal bacteria using GT rotary instrumentation, 5.25% NaOCl, EDTA, and $Ca(OH)_2$. J Endod 2005;31:359–63.
14. Usman N, Baumgartner JC, Marshall JG. Influence of instrument size on root canal debridement. J Endod 2004;30:110–2.
15. Rollison S, Barnett F, Stevens R. Efficacy of bacterial removal from instrumented root canals in vitro related to instrumentation technique and size. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2002;94:366–71.
16. Dalton BC, Orstavik D, Phillips C, Pettiette M, Trope M. Bacterial reduction with nickel-titanium rotary instrumentation. J Endod 1998;24:763–7.
17. Card SJ, Sigurdsson A, Orstavik D, Trope M. The effectiveness of increased apical enlargement in reducing intracanal bacteria. J Endod 2002;28:779–83.
18. Siqueira J, Araujo M, Garcia P, Fraga R, Saboia Dantas C. Histological evaluation of the effectiveness of five instrumentation techniques for cleaning the apical third of root canals. J Endod 1997;23:499–02.
19. Tan BT, Messer HH. The quality of apical canal preparation using hand and rotary instruments with specific criteria for enlargement based on initial apical file size. J Endod 2002;28:658–64.
20. Wu M, Wesselink R. Efficacy of three techniques in cleaning the apical portion of the curved root canals. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1995; 79:492–6.
21. Weiger R, El Ayouti A, Lost C. Efficiency of hand and rotary instruments in shaping oval root canals. J Endod 2002;28:580–3.
22. Weine F. Endodontic therapy. St. Louis: C.V. Mosby, 1972:209–22.
23. Contreras MA, Zinman EH, Kaplan SK. Comparison of the first file that fits at the apex, before and after early flaring. J Endod 2001;27:113–6.
24. Tan BT, Messer HH. The effect of instrument type and preflaring on apical file size determination. Int Endod J 2002;35:752–8.
25. Pecora JD, Capelli A, Guerisoli DM, Spano JC, Estrela C. Influence of cervical preflaring on apical file size determination. Int Endod J 2005;38:430–5.
26. Coldero LG, McHugh S, MacKenzie D, Saunders WP. Reduction in intracanal bacteria during root canal preparation with and without apical enlargement. Int Endod J 2002;35:437–46.
27. Baugh D, Wallace J. The role of apical instrumentation in root canal treatment: a review of the literature. J Endod 2005;31:333–40.