# Influence of Apical Preparation Size on Effective Conventional Irrigation in the Apical Third: A Scanning Electron Microscopic Study

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## ABSTRACT

**Objective:** This study aimed to investigate the relationship between final apical preparation size and smear layer removal in the apical third using conventional irrigation in mandibular bicuspids.

**Methods:** A total of 66 extracted human mandibular bicuspids with comparable root canal morphology were equally divided into five experimental groups and a control group (n=11). Based on their experimental group, samples were instrumented up to size 25, 30, 35, 40, or 45 with 0.04 taper using 2.5% sodium hypochlorite (NaOCl) as the irrigant. Final irrigation was performed with 17% EDTA followed by 2.5% NaOCl. The control group was instrumented up to size 45/.04, and was irrigated with distilled water. Samples were sectioned buccolingually, and they were qualitatively evaluated under scanning electron microscope for efficacy in smear layer removal. The Kruskal–Wallis and Mann–Whitney U tests were employed for statistical analysis.

**Results:** Statistical analysis revealed comparable removal of smear layer in the coronal and middle thirds of all experimental samples regardless of grouping. When using file size larger than 35, removal of smear layer in the apical third significantly improved (p<0.05). No significant difference was observed between 40/.04 and 45/.04.

**Conclusion:** Under the conditions of this study, using conventional irrigation, apical enlargement more than size 35/.04 is essential to enhance removal of smear layer at the apical third.

**Keywords:** Apical diameter, conventional irrigation, dentinal tubules, sodium hypochlorite, smear layer, SEM

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## HIGHLIGHTS

- Apical preparation size of at least 40/.04 is required to enhance the debridement of the apical third.
- Increasing apical diameter to 45/.04 does not result in more removal of smear layer at the apical third.
- Using the right needle size combined with optimal apical preparation size is essential for effective irrigant replacement particularly at the apical third.

## INTRODUCTION

The primary goal of endodontic therapy is removal of lesion etiology via effective chemomechanicalcleaning and shaping of root canal system. The morphology of radicular structures, however, does notlend itself to a simple and effective instrumentation. Exceptional difficulty is associated with thorough debridement and disinfection of the apical third (1). Historically, several standardized protocols have been presented with regards to the ideal apical enlargement to ensure effective apical debridement, such as enlarging of canals to a size three files greater than initial binding (2). However, the first file that binds does not always correlate with the true apical dimension. The circumferential dentine removal sufficient to render the canal inert may not be achieved in this manner (3). Alternatively, several authors advocate for minimal apical enlargement to conserve unaffected tooth structure and limit various sequalae (4). The selection of master apical file size and enlargement of root canal systems is ultimately the responsibility of the clinician, who must weigh several factors to determine the most appropriate protocol for the individual patient.

Independently, neither mechanical nor chemical debridement is likely to achieve a clinically acceptable result, which has led to discussion of clinical minimums in both aspects for therapeutic success (5). To deliver chemical agents to the most apical and inaccessible extents of the canal space, the canal must be instrumented up to a certain size. The exact size requirement is a widely debated topic. Numerous researchers argue for and against a standardized protocol (6, 7). Inadequate removal of biologic load may preclude successful endodontic therapy, requiring additional intervention and potentiating more complications (8). Inadequate debridement of specifically the apical third has been linked to significantly poor prognoses (9). The smear layer, an amorphous layer of

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dentine and bacterial debris, obstructs the development of an adequate apical seal, which may allow for radicular reinfection (10). The chelating agents such as EDTA are used specifically to remove this film, but their ability to reach and remove materials may be hampered by insufficiently prepared canals (11). Therefore, the clinician must enlarge the canal space to a sufficient degree to allow adequate chemomechanical instrumentation into all areas of the root canal system, including the apical third.

Overly aggressive instrumentation, however, can also contribute to sequelae or failure of endodontic therapy in a number of manners. Extrusion of materials beyond the apical foramen causes postoperative inflammation, lengthening of healing times, or even failure of treatment (12). The remaining radicular structure will be used to support a future restoration, and typical forces of occlusion may lead to fracture in scenarios of reducing dentinal thickness (13). Excessive canal shaping may also contribute to operative complications such as perforation, ledging, and transportation (14). While effective debridement of radicular spaces is paramount in endodontic therapy, overzealous efforts to this end may lead to severe complications.

This study used the SEM imaging of the radicular walls following in-vitro root canal treatment of extracted human teeth. Adequately debrided surfaces were expected to have open dentinal tubules, indicative of the irrigant's ability to reach the area of observation. Although in this investigation, the debridement of the apical third was of special interest, coronal and middle thirds were also discussed to reveal the efficacy of this irrigation protocol on them.

The objective of this study was to investigate the relationship between final apical preparation size and smear layer removal in the apical third using conventional irrigation in mandibular bicuspids.

## MATERIALS AND METHODS

For this study, 66 extracted human mandibular single-rooted teeth with a single canal were selected. Similarities in radicular morphology, including canal length, shape, and flare, were radiographically confirmed. Exclusion criteria included the following: cracked, restored, carious teeth; presence of more than one canal; immature root apex; previous root canal therapy; apical diameter larger than size 15 K-type file; and canal calcification. Approval from the ethics committee was obtained (#EA/95/14). After developing an access preparation, size #10 K-type files were extruded just beyond the apical foramen, then withdrawn 1 mm to determine working length (WL). Canals were instrumented to size 20/.02 to establish a glide path. Root ends were sealed with flowable composite (Permaflo, Ultradent, USA) to prevent irrigants from escaping through the apex. Specimens were ran-domly divided into five test groups and a control (n=11).

All canals were instrumented with EndoSequence rotary NiTi instruments (Brasseler, Savannah, GA, USA) in a standardized crown down manner to final apical sizes of 25, 30, 35, 40, and 45. All groupings used a taper of 0.04. In the test groups, canals were manually irrigated after each instrument change using 2 mL NaOCl (2.5%) in a syringe with a 30-gage side vented needle (ProRinse, DENTSPLY, Tulsa Dental Specialties, TN, USA). Fi-

nal irrigation of each specimen consisted of 2 mL EDTA (17%) (Inter Med-Vista Dental. Racine, WI, USA), which remained in the canal for 1 min, followed by 5 mL NaOCl (2.5%) using the aforementioned needle. In the control group, canals were instrumented to the largest size used in the test group (45/.04), and irrigated using only distilled water. In all specimens, the needle tip was introduced into the canal until binding occurred followed by 1 mm withdrawal to facilitate outward flow of the irrigant. After preparation and during final irrigation, the needle was inserted 1 mm short of the WL.

### Root sectioning and scanning electron microscopy imaging

Following cleaning and shaping, root canals were dried with paper points (Brasseler, Savannah, GA, USA). The coronal orifices were closed with cotton pellets and Fuji TRIAGE (GC Corporation, Tokyo, Japan) to prevent entry of debris into canals during sectioning. Grooves were cut buccolingually along the long axis of the tooth with an STX-202A diamond wire saw (MTI corpo-ration, Richmond, CA, USA) without penetrating the root canal space. Using a chisel in the created indentation, the specimen roots were split down the length of the root canal into two equal halves. The mesial portion was retained for further evaluation. Samples were dehydrated in a graded series of ethanol solu-tions (30–100%), coated with gold, and viewed with a scanning electron microscope (Amray, Bedford, MA, USA) at 15 kV.

### SEM evaluation and statistical analysis

The representative SEM images taken from the center of the coronal, middle, and apical regions of each canal were observed at 1000x magnification. A five-point scale (Table 1), described in Caron et al. (10), was used to evaluate the presence or absence of smear layer, with gradations seen in Table 1. All comparisons were made using images at the same magnification.

Two trained and blinded evaluators independently rated each masked fragment. Evaluators had no prior knowledge of the cleaning and shaping procedures, and were well acquainted with qualitative analysis of the SEM root canal images. When evaluator scores disagreed, the lower score was taken.

### Statistical analysis

Data were analyzed via the Kruskal-Wallis and Mann-Whitney U tests with the SPSS 17 statistical software. Each grouping consisted of 11 samples (n=11), totaling 66 across all groupings. A difference of p<.05 was considered significant. Cohen's kappa was calculated to evaluate the interobserver inconsistencies.

**TABLE 1.** The scoring system used to evaluate the presence or absence of smear layer

| Scores | Criteria |
|---|---|
| Score 1 | No smear layer and dentinal tubules open. |
| Score 2 | Small amounts of scattered smear layer and dentinal tubules open. |
| Score 3 | Thin smear layer and dentinal tubules partially open (characteristic crescent presentation). |
| Score 4 | Partial covering with a thick smear layer. |
| Score 5 | Total covering with thick smear layer. |

## RESULTS

Scores of the smear layer are listed in Table 2. The mean values and standard deviation of smear layer scores for experimental groups at each of the root canal thirds are shown in Table 3. All experimental groups showed significantly less smear layer than the control group did in all thirds of the root canals (p<0.05). The apical third showed significantly more smear layer than the coronal and middle thirds (p<0.05) did. No significant dif-ference was found between the coronal and middle thirds in all experimental groups (p>0.05). In the apical third, increasing the diameter of the canal preparation decreased the amount of smear layer left on the canal walls (Tables 2 and 3, Fig. 1, Fig. 2). Differences in smear layer in the apical third between groups 40 and 45 were not statistically significant (p>0.05), nor were between groups 25, 30, and 35 (Table 3). Groups 40 and 45 showed significantly less smear layer at the apical third than all other groups (p<0.05) did. The surfaces of root canals and the dentinal tubules in the apical third of samples in groups 25, 30, and 35 were partially covered with thin smear layer and partially opened dentinal tubules (Fig. 3). Examination of the surface of root canal walls in the control group showed the presence of a thick smear layer covering the entire length of the root canals (Fig. 4). The interobserver differences never ex-ceeded 1 score, and Cohen's kappa exceeded 0.85 (κ>0.85).

## DISCUSSION

The chemomechanical debridement of radicular canals to clinically satisfactory levels is paramount in the success of endodontic therapy. Therefore, it necessitates an understanding of methods to achieve the aforementioned level of smear layer removal. This study found no significant difference in smear layer removal in the coronal or middle thirds of any pairings. So, the analysis of this study will instead focus on the smear layer in the apical third. Within the apical third, a significant difference was found in the smear layer removal of radicular walls in groups instrumented to file size 40 or 45 (p<0.05). This significant difference may most likely be attributed to improved ability to irrigate the apical third, facilitating chemomechanical debridement. The comparison to the control group, in which distilled water was used as the primary irrigant instead of sodium hypochlorite, further demonstrates that it is the im-

**TABLE 2.** Summary of scores for smear layer

| Group | Coronal third scores | | | | | Middle third scores | | | | | Apical third scores | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 1 | 2 | 3 | 4 | 5 | 1 | 2 | 3 | 4 | 5 |
| 25 (25/04) | 8 | 3 | 0 | 0 | 0 | 4 | 5 | 2 | 0 | 0 | 0 | 2 | 5 | 2 | 2 |
| 30 (30/04) | 9 | 2 | 0 | 0 | 0 | 8 | 3 | 0 | 0 | 0 | 0 | 3 | 4 | 4 | 0 |
| 35 (35/04) | 7 | 4 | 0 | 0 | 0 | 3 | 6 | 2 | 0 | 0 | 1 | 3 | 4 | 3 | 0 |
| 40 (40/04) | 6 | 4 | 1 | 0 | 0 | 1 | 9 | 1 | 0 | 0 | 4 | 6 | 1 | 0 | 0 |
| 45 (45/04) | 11 | 0 | 0 | 0 | 0 | 9 | 2 | 0 | 0 | 0 | 5 | 5 | 1 | 0 | 0 |
| Control (45/04) | 0 | 0 | 0 | 6 | 5 | 0 | 0 | 0 | 7 | 4 | 0 | 0 | 0 | 4 | 7 |

**TABLE 3.** Mean and standard deviation of the smear layer scores of each experimental group

| Group | 25 | | 30 | | 35 | | 40 | | 45 | | Control | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| Apical | 2.6[a, a] | 0.8 | 1.7[a, a] | 0.6 | 2.9[a, a] | 0.8 | 1.7[a, b] | 0.9 | 1.6[a, b] | 0.7 | 4.4[a, b] | 0.7 |
| Coronal | 1.3[b, a] | 0.4 | 1.2[b, a] | 0.4 | 1.4[b, a] | 0.5 | 1.5[b, a] | 0.7 | 1.0[b, a] | 0.0 | 4.1[a, b] | 0.8 |
| Middle | 1.8[b, a] | 0.7 | 1.3[b, a] | 0.5 | 1.9[b, a] | 0.7 | 2.0[b, a] | 0.4 | 1.2[b, a] | 0.4 | 3.7[a, b] | 0.8 |
| P value | 0.0003 | 0.04 | 0.0001 | 0.0001 | 0.008 | 0.17 | | | | | | |

The first superscript letter indicates significance within columns, where means with the same letter are not significantly different at p≤0.05. The second superscript letter indicates significance within rows, where means with the same letter are not significantly different at p≤0.05
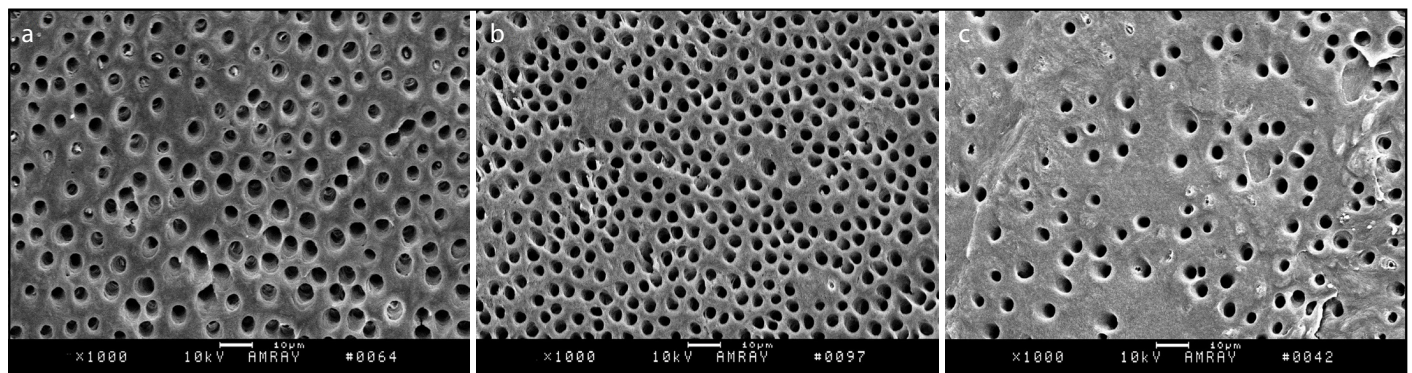


**Figure 1.** Scanning electron photomicrographs representative of group 40 (40/04), showing clean root canal at (a) coronal, (b) middle, and (c) apical thirds. Magnification: 1000x
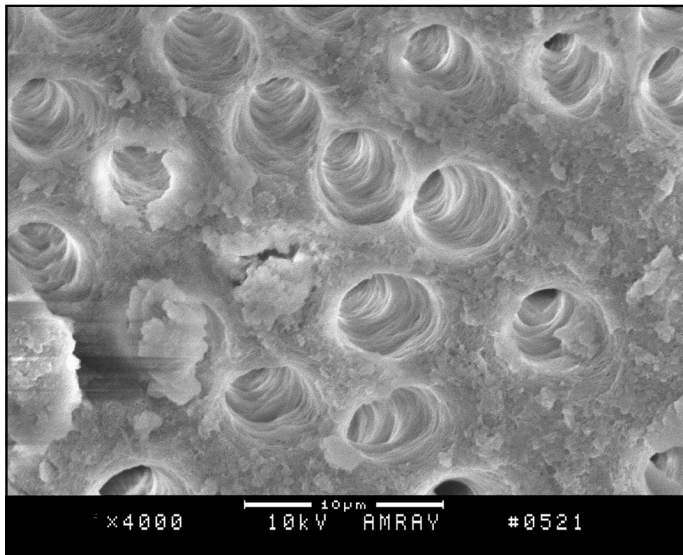
**Figure 2.** Scanning electron photomicrograph showing presence of debris blocking some dentinal tubules in the apical third 4000x

proved access to the apical third by effective irrigants, and not mechanical debridement alone that allows adequate smear layer removal.

This study used the smear layer to evaluate the cleaning efficacy of the different groupings. While some authors have found comparable obturation with and without removal of the smear layer, thereby suggesting its irrelevance, other reports demonstrate removal of smear layer to results in increased surface area for

adhesive binding and a decreased chance for reinfection of the canal space following treatment (15). Selection of irrigants was based on efficacy of material and prevalence in clinical practice. NaOCl is the most used irrigating solution for root canal therapy. EDTA, a chelating agent, is a common irrigant used for the elimination of the smear layer (16). Together, these irrigants have shown ability to render the canal space inert when used in conjunction with mechanical debridement (17).

Proponents of larger apical enlargement suggest that this is the most predictable method to clean and disinfect radicular structures (18). Numerous studies proved that larger preparation size allows both enhanced irrigation in remote areas and greater reduction in remaining bacteria and dentinal debris (19, 20). Huang et al. found that increased apical size and taper allowed enhanced irrigation in all areas of the root canal system (21). Furthermore, larger instruments may be employed to improve contact with canal walls, thereby producing more efficacious cleaning (6). Usman et al. found a significant difference in smear layer removal between size 20 and size 40 GT instruments, and importantly, that it was the master apical file size and not the number of irrigation cycles that contributed to improved smear layer removal (7). Following cleaning and shaping, increased apical enlargement is associated with improved obturation results (22). Thus, it can be understood that a certain minimum threshold of apical enlargement is required for effective chemomechanical debridement.

To determine the minimum apical preparation size and taper for effective irrigation, a computational fluid dynamic model
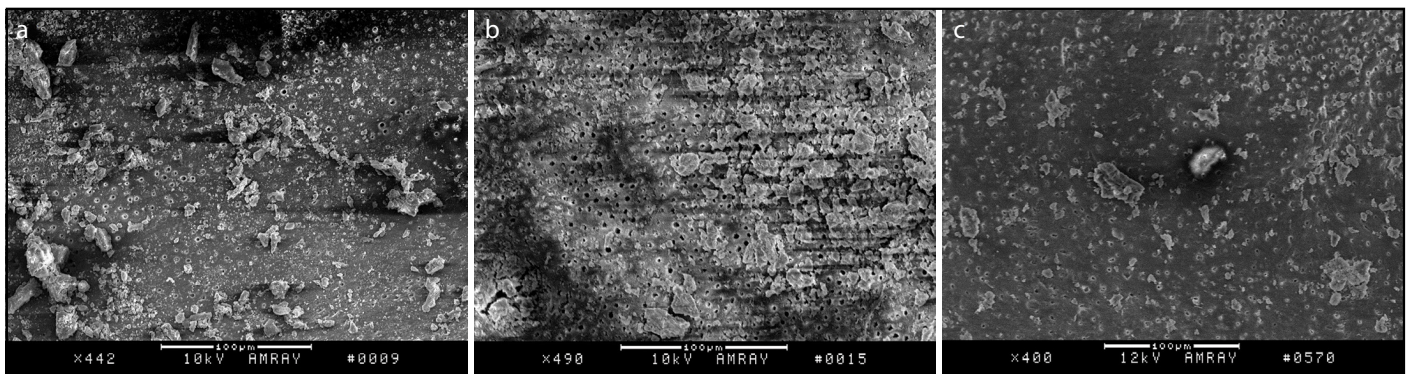


**Figure 3.** Scanning electron photomicrographs showing thin smear layer and dentinal tubules partially open at apical thirds of groups 25 (a), 30 (b) & 35 (c). Magnification: 400x
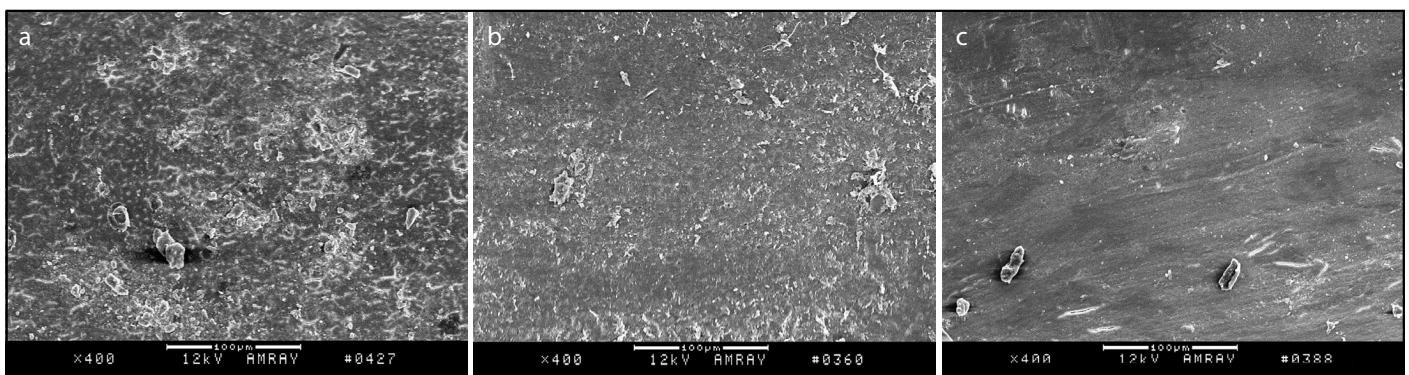


**Figure 4.** Scanning electron photomicrographs showing total surface covering with thick smear layer in control group in Coronal (a), Middle (b) and Apical (c) thirds. Magnification: 400x

was employed by Boutsioukis et al. (20, 23), and concluded that apical enlargement to size 25 or greater improved the efficacy of conventional irrigation (20, 23). Furthermore, while increased apical taper was found to improve irrigation and shear wall stress, minimally tapered canals with larger apical preparation sizes were found to have more favourable irrigation profiles relative to canals with smaller apical preparation sizes and increased taper (20).

The associated sequalae are the counterpoints to the benefits of increased apical enlargement. Borges et al. investigated the removal of smear layer in the apical third of mildly to moderately curved canals with instrumentation of 30/.02 compared to 45/.02 and found no difference. They concluded that the removal of debris from the apical third does not depend on the final instrument diameter used (24). They, however, observed increased extrusion of debris through the apical foramen in the 45/.02 group, which may lead to postoperative periapical inflammation and delayed healing (24). To improve contact with and effectively plane canal walls, the use of increasingly larger file sizes may be misguided, as no apical file can adapt to the intricate radicular anatomy to completely clean root canal walls (6). Furthermore, provided adequate irrigation, such contact may be unnecessary, and would only result in increased loss of radicular dentine (25). Increased apical preparation sizes also produce a higher incidence of iatrogenic complications such as perforation, canal transportation, and ledging (2). A recent study showed that under vertical and lateral occlusal loads, greater apical enlargement resulted in increased stress on the remaining dentinal walls, especially at the apex. It also suggested that these areas would therefore be at an increased risk for crack formation during obturation (26). Overly aggressive canal preparation, such that the apical constriction is lost, may allow extrusion of irrigant solutions beyond the apex, which may cause breakthrough pain, mucosal and bone necrosis, sensory and motor defects, and may require hospitalization (27). While increased instrumentation may allow more efficacious cleaning, the use of increasingly larger files sizes should be avoided where appropriate use of an irrigant alone would suffice.

Instead of a standardized final apical file size, many authors recommend employing the Grossman criterion of expanding the canal to at least three sizes beyond the first file to bind to WL (28). Use of this method is predicated on techniques such as adequate coronal and middle third preflaring, which significantly improve determination of first file to bind (2).

This study has some limitations that should be addressed, and further research is needed. This study employs only the straight canals, which does not reflect the plethora of anatomical variants the clinician may encounter. Ideal apical enlargement of a significantly curved or multi-canal tooth may require enlargement not herein described. The efficacy of cleaning was determined based on the number of opened dentinal tubules, but pathogens may exist in unobserved areas deeper within the dentinal matrix. Pathogens such as Enterococcus faecalis can extend as far as 1100 μm into dentinal tubules, and penetration of irrigant may be limited to 160 μm (29). Therefore, presence of non-occluded tubules assists but does not ensure the eradication of pathogens within the dentinal tubules via irrig

ant solutions. Finally, irrigants were not activated in this study, an adjunct that is becoming increasingly more popular (30). Further research incorporating the aforementioned elements is warranted, and may be pursued in the future.

## CONCLUSION

Apical enlargement more than size 35/.04 with conventional irrigation allowed enhanced smear layer removal in the apical third.

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