Original Article

Effect of “Apical Clearing” and “Apical Foramen Widening” on Apical Ramifications and Bacterial Load in Root Canals: An *Ex-vivo* Stereomicroscopic Study

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Received 12 June, 2013/Accepted for publication 25 November, 2013

Abstract

The purpose of the study was to determine the effect of apical clearing and apical foramen widening in reducing apical ramifications and bacterial load in the apical third of root canals. The mesio-buccal roots of 21 maxillary first molar teeth were inoculated with *Enterococcus faecalis* suspension using a sterile pipette. Samples were incubated at 37°C for 72 hrs and divided into 3 groups: Group A, control group (n = 5), no preparation; Group B (n = 8) conventional preparation alone; and Group C (n = 8), apical clearing and foramen widening in addition to conventional preparation. Bacterial counts were semi-quantitatively analyzed pre- and post-preparation. Samples were demineralized with 5% nitric acid after injection of India ink. Cross sections were obtained at every 0.5 mm from the apex to 3 mm of the root using a vibratome and viewed under a stereomicroscope at 64× magnification to locate any debris or apical ramifications. The Kruskal-Wallis and Mann-Whitney U tests were used for the statistical analysis. A statistically significant difference was observed (p value 0.006) in the number of ramifications among the 3 groups. Group C had a lower average number of ramifications (1) than Group B (2.5) or A (4). The debris score was analyzed at each level (0.5–3 mm). A statistically significant difference was observed at 0.5 mm and 1 mm between Group A and C (p = 0.0041) and Group B and C (p = 0.0050), whereas no difference was found between Group A and B (p > 0.05). These results indicate that there was less debris and fewer apical ramifications in Group C. The microbiological study revealed a lower number of colony forming units (10^2 – 10^3) in Group B or C than in Group A ($>10^6$). These results suggest that apical widening and clearing facilitates removal of apical ramifications and bacterial load within root canals.

Key words: Apical clearing — Apical foramen widening — Apical ramifications

Introduction

The success of endodontic treatment largely depends on thorough chemo-mechanical debridement. Mechanical preparation requires the appropriate instrumentation to allow bacteria to be removed from the root canal system. Complete sterility, however, is difficult to achieve by mechanical means alone, and any residual debris left over following such preparation may lead to treatment failure¹⁴⁾. Such residual bacteria are mainly found in ramifications, most of which are present in the apical third of the root canal^{21,22)}.

Lateral canals and apical ramifications can make some areas of the root canal inaccessible to instruments^{9,23)}. Moreover, such areas can harbor significant numbers of bacteria, providing them with easy access to periradicular tissue, which then leads to the development of disease^{11,14,16)}. Such areas are difficult to reach, clean, disinfect, and fill during treatment. It has been suggested that more emphasis on chemo-mechanical preparation is needed to remove such ramifications and decrease the bacterial load to the point where root canal failure can be avoided¹⁹⁾. Preparation of the root canal beyond the apical constriction and apical enlargement remain controversial, however. "Apical clearing" and "apical foramen widening" are further options following canal preparation. These procedures are intended to maximize debridement and irrigation and increase the size of the apical preparation in small canals, allowing for procedural errors to be minimized²⁰⁾.

Apical clearing was introduced by Walton and Torabinejad²⁵⁾, who recommended the sequential use of files two-to-four sizes larger than the master apical file and reaming throughout the working length. This procedure is used to clear away remaining debris and slightly enlarge the apical preparation. After a final irrigation and drying, the canal is then reamed once again with the largest apical file to remove any impacted dentin debris, thus clearing the canal. Apical foramen widening involves preparing the root canal beyond the apical constriction to allow

debridement of the cemental canal and major apical foramen.

There is very little evidence from *in vitro* studies to show that apical clearing and foramen widening reduces bacterial load. Studies on experimental animals, however, have demonstrated that it improves periapical healing³⁾ and repair^{2,7)}. To the best of our knowledge, no studies to date have shown a direct correlation between apical clearing and foramen widening and a decrease in bacterial load and apical ramifications in the root canal. Therefore, the purpose of this study was to investigate the effect of apical clearing and foramen widening in reducing apical ramifications and bacterial load in root canals.

Materials and Methods

Twenty-one freshly extracted permanent first and second maxillary molars from patients aged between 15 and 30 years were used. The teeth were divided into the following 3 groups: Group A, in which no preparation was carried out (n=5); Group B, in which only conventional endodontic preparation was performed (n=8); and Group C, in which apical clearing and the foramen widening technique were employed¹⁷⁾ (n=8). All the teeth were decoronated at the cemento-enamel junction using a diamond disc in an air rotor hand-piece under copious water cooling. The roots were placed in a 5.25% sodium hypochlorite solution for 48 hrs to clean any remaining pulp debris in the canal. The working length was determined by passing a non-cutting instrument through the canal until it became visible at the apical foramen, after which the length was reduced by 1 mm (under magnification). All the samples were sterilized by application of ethylene oxide gas overnight.

A suspension of bacteria was prepared by adding 1 ml pure culture of *Enterococcus faecalis* grown in brain heart infusion broth. All the teeth were completely filled with *E. faecalis* suspension using a sterile pipette. A sterile K

Fig. 1 Diagram depicting **i)** infected root without preparation showing abundant bacteria in canal and ramifications and infected cementum; **ii)** after conventional root canal preparation, untouched ramifications and necrotic cementum beyond apical constriction may still harbor bacteria; **iii)** after apical clearing and foramen widening technique, very few ramifications and cleaned cemental canal.

file was used to apply the bacterial suspension to the apical foramen. The canal orifices were sealed with gutta percha and the apical third of the root canal with 2 coats of varnish. The samples were then incubated at 37°C for one week. Preoperative microbiological samples were taken at this stage.

No preparation was carried out in Group A (Fig. 1-i). In Group B (Fig. 1-ii), all the samples were subjected to conventional endodontic preparation only (crown-down method), with apical preparation 3 sizes larger than the initial binding file. In Group C (Fig. 1-iii), crown-down preparation was performed while maintaining apical patency with a #10 K file, after which the root canals were prepared until apical constriction was 2–4 sizes greater than the initial master apical file (apical clearing). The cemental canal was then penetrated with a #15 K file and the major apical foramen located. The apical foramen was prepared up to a #25 K file (apical foramen widening)³⁾. The conventional irrigation protocol (2 ml of 5.25% sodium hypochlorite with a safe-handed 27-gauge needle at each instrument change and the total preparation time standardized to 10 min per specimen) was used⁶⁾ throughout the procedure in groups B and C. After canal preparation and rinsing with 17% EDTA solution, a final rinse with phosphate-buffered normal saline was performed and microbiological samples obtained with a micropipette.

The samples were streaked onto Mueller Hinton Agar plates and incubated at 37°C for 72 hrs. Colony forming units were counted semi-quantitatively and recorded for each sample. After taking samples for microbiological evaluation, India ink was injected into all the roots using a micropipette. Demineralization was performed in acid buffer solution (5% nitric acid) for 4 days, with the solution being replenished every day. Cross sections 500 μ m in thickness were obtained at every 0.5 mm up to 3 mm of the apical third of root by using a vibratome. These sections were examined for apical ramifications (Fig. 2) and debris (Fig. 3) under a stereomicroscope at 64 $\times$ magnification and the results compared between each group.

The apical ramifications were counted with the photographs of each section of the tooth at each level (0–3 mm) side by side so that no repetition occurred. The debris score was calculated according to the following scale: 0, no debris; 1, <½ of the canal wall covered with debris; 2, $\geq$ ½ of the canal wall covered with debris; and 3, all the canal wall covered with debris.

The Mann-Whitney U and Kruskal-Wallis tests were used for the statistical analysis, which was carried out using the Stata 11.0 software package (Stata Corp., College Station, Texas, U.S.A.). The data are presented as the median (min–max). A p value of <0.05 was considered as statistically significant.

Fig. 2 Tooth sections showing accessory canal (AC), fins (F), isthmus (Is), apical delta (AD) and accessory foramen (AF)

Fig. 3 Tooth sections showing debris
I and II at 0.5 mm and 1 mm from apex, respectively. **a**-control group, **b**-conventional group, and **c**-apical clearing and foramen widening group.

Table 1 Statistical analysis of number of CFUs between groups B and C

Bacterial count (in CFUs)	Group B (Conventional)	Group C (Apical clearing and widening)	p value	
			Overall	B vs. C
Median (Min–Max)	9,000 (1,000–13,000)	350 (100–1,200)	0.0002	0.0016

Table 2 Statistical analysis of number of apical ramifications among groups

Apical ramifications	Group A	Group B	Group C	p value			
	n = 5	n = 8	n = 8	Overall	A vs. B	A vs. C	B vs. C
Median (Min–Max)	4 (3–5)	2.5 (2–8)	1 (0–3)	0.005	0.45	0.003	0.01

Table 3 Statistical analysis of debris scores at each level

	Group A	Group B	Group C	p value			
	n = 5	n = 8	n = 8	Overall	A vs. B	A vs. C	B vs. C
0.5 mm	3 (2–3)	3 (1–3)	1 (0–2)	0.003	0.8	0.004	0.005
1 mm	3 (1–3)	2 (1–3)	0 (0–1)	0.0008	0.09	0.004	0.0008
1.5 mm	3 (1–3)	1.5 (0–3)	0.5 (0–2)	0.015	0.11	0.01	0.06
2 mm	3 (1–3)	1 (0–2)	0 (0–2)	0.019	0.078	0.013	0.08
2.5 mm	3 (2–3)	1 (0–3)	1 (0–2)	0.007	0.01	0.004	0.28
3 mm	3 (1–3)	1 (0–3)	0 (0–1)	0.004	0.05	0.004	0.02

Results

Preoperative bacterial counts performed for a semi-quantitative analysis revealed confluent growth ($>10^5$), while postoperative bacterial counts between the two techniques varied (Table 1). The median postoperative bacterial count in Group B was much higher, at 9×10^3 $\{(1-13) \times 10^3\}$, than that in Group C, 3.5×10^2 $\{(1-12) \times 10^2\}$. The median number of apical ramifications (Table 2) was 4 (3–5), 2.5 (2–8), and 1 (0–3) in Groups A, B, and C, respectively. A statistically significant difference was observed in decrease in number of apical ramifications between Groups A and C and Groups B and C, whereas no significant difference was observed between Groups A

and B. The debris score (Table 3) was lower in Group C, at 3.5 (0–7), than in Group A, at 17 (11–18), or B, at 9 (5–15). No significant difference was observed in the total median debris score between Groups A and B.

Discussion

The apical area is the critical zone for instrumentation^{8,18}. Ramifications can be observed anywhere along the length of the root, but occur more frequently in the apical portion and in the posterior teeth⁵. The treatment outcome will be poor if these anatomical anomalies are not identified, prepared, and obturated. Seventy percent of

cases of refractory apical periodontitis had significant apical ramifications in the apical third of the root apex of teeth²⁴. This suggests a close relationship between the anatomic complexity of the root canal system and the persistence of periradicular pathosis.

The two primary mechanical elements involved in biomechanical preparation are the apical width and apical limit of debridement. Traditionally, canal preparation extends as far as the apical constriction, which represents the point at which the diameter of the canal is narrowest^{8,18}. Moreover, it is believed that the master apical file should be 3 times larger than the first binding file²⁶. This recommendation has now been called into question, however, as this does not correlate with the true apical constriction, and it is unclear as to whether enlarging by 3 sizes will adequately remove dentin circumferentially from the root canal walls. On the other hand, minimal apical enlargement has been suggested to conserve tooth structure and limit extrusion of filling materials⁴. One study has suggested that a large diameter at the coronal orifice and a gradual taper towards the apical constriction offers the ideal instrumentation technique for thermo-plasticized obturation³¹. Indeed, many instrumentation techniques have been designed with the obturation phase in mind, rather than achieving optimal chemo-mechanical debridement of the infected root canal systems¹.

Apical clearing (a) removes existing and created dentin chips and soft tissue debris compacted in the apical region; (b) permits deeper placement of an irrigation needle for more effective final irrigation; and (c) enlarges and produces a more uniform shape in the apical region in order to facilitate placement and fit of the master cone¹². Apical foramen widening, on the other hand, is believed to facilitate healing by removing infected cementum and any newly deposited cementum or newly grown connective tissue that may have sealed off the apical foramen or accessory canals inside the apical root canal^{2,3}.

In teeth with chronic periapical lesions,

microorganisms are present in the apical delta, ramifications, dentinal tubules, and cementum³. Therefore, performing apical widening removes a greater amount of bacteria and promotes more favorable conditions for healing.

Longitudinal studies have shown that instrumentation with larger file sizes does not significantly enhance the success of endodontic therapy. However, such studies have often been retrospective or included confounding factors, which has rendered their results inconclusive¹. One recent clinical study found a correlation between apical width and the success of root canal treatment; an improvement in outcome was observed with increase in apical enlargement. No statistically significant difference was found, however, with enlargement beyond three sizes above that of the initial binding file¹⁵. Evidence of a higher success rate when proper cleaning is carried out before obturation indicates the importance of apical clearing and foramen widening. Since periapical tissue has the potential to heal, initial treatment of periapical lesions should solely be directed towards the removal of causative factors. Root canal treatment is based primarily on the removal of microbial infection from the complex root canal system.

The maxillary molars have an intricate anatomical configuration. A high incidence of accessory canals and apical ramifications (76.7%) was found in the maxillary first molars³⁰, and the morphology of the mesio-buccal root canal, in particular, shows wide variation. Treatment of the mesio-buccal canal is, therefore, very difficult, and various methods have been used to elucidate its morphology³⁰.

Therefore, in the present study, the mesio-buccal roots of maxillary molars with a similar anatomy were chosen. The teeth were obtained from patients of similar age to standardize the size of the apical foramen and length of the cemental canal. Decoronation was performed to facilitate collection of microbiological samples and chemo-mechanical preparation. The samples were placed in 5.25% sodium

hypochlorite to remove any vital or necrotic pulp debris and sterilized to remove any residual bacteria. All the samples were filled with *E. faecalis* and incubated for 72 hrs before confirmation of bacterial growth. Chemo-mechanical preparation was performed under sterile conditions and a standardized protocol followed.

There are various methods for studying the morphology of human permanent teeth: use of radiographs¹³⁾, cutting the teeth at different levels²⁸⁾, making polyester resin cast replicas of the pulp space²⁰⁾, clearing and injection of dye^{27,29)}, and micro-CT³⁰⁾. Since the apical ramifications are very small and extend from the main canal in a branching pattern, sectioning was selected here. Sections were obtained from the apex up to 3 mm of the root canal, as the maximum number of apical ramifications (98%) and accessory canals (93%) is contained within the apical 3 mm of the root¹⁰⁾, and observations performed under a stereomicroscope at 64× magnification.

The results showed that conventional preparation was ineffective in removing apical ramifications, whereas apical clearing and apical widening yielded a significant decrease in the number of apical ramifications, which potentially harbor bacteria, even after completion of conventional cleaning and shaping procedures.

Evaluation of the overall debris scores revealed that there was much less debris in Group C than in Group A or B. When compared section-wise, a strongly significant difference was observed in the scores at the 0.5 and 1 mm levels between Group C and B. This difference may have been due to apical widening facilitating cleaning of the cemental canal. At the other levels, the debris scores showed a decrease, although not statistically significant, due to final apical reaming (apical clearing).

Siqueira Jr and Roças¹⁹⁾ have shown a correlation between positive culture and a poor prognosis. Therefore, the goal of endodontic treatment should be to reduce the bacterial population to levels that would not be detected by culture procedures (arguably, $<10^3$ – 10^4

cells). The results of the present study conform to the recommended level of microbial populations, that is, below 10^3 – 10^4 cells.

During the mechanical instrumentation of root canals, debris may get impacted in the narrow, apical area of the canal. If not removed, it can provide a culture medium for bacteria to thrive. There are different views regarding the cleaning of apical debris, whether by mechanical means or chemical agents. The results of the present study indicate that apical clearing and foramen widening as an additional step after conventional cleaning and shaping of the root canal improves removal of apical ramifications and debris. The efficacy of this technique in the management of periapical lesions was recently reported in a clinical study¹⁷⁾.

Conclusion

The following conclusions were drawn from the present study.

1. Modified root canal preparation technique employing apical clearing and apical foramen widening was very effective in removing apical ramifications. Small ramifications branching from the main canal were included within the lumen of the main canal and loose debris was removed.

2. A significant decrease was observed in colony forming units with this modified technique in comparison with conventional preparation alone, confirming that it provides better debridement of the root canal.

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