Influence of Different Apical Preparations on Root Canal Cleanliness in Human Molars: a SEM Study

Gianluca Plotino[1], Nicola M. Grande[1], Luigi Tocci[1], Luca Testarelli[1], Gianluca Gambarini[1]

[1]Department of Endodontics, "Sapienza" University of Rome, Italy.

Corresponding Author:
Gianluca Plotino
Via Calabria 25 - 00187 Rome
Italy
E-mail: endo@gianlucaplotino.com

ABSTRACT

Objectives: To compare the influence of type and dimensions of the apical preparation on the cleanliness of the apical area in molars.
Material and Methods: A total of 120 root canals (MB and DB root canals from 30 maxillary molars and mesial root canals from 30 mandibular molars) were instrumented with M*two* NiTi rotary instruments to a size 25/0.06 taper and were equally divided into three different experimental groups depending on the subsequently apical root canal preparation: Group 1: no further apical preparation, Group 2: apical preparation with M*two* files to a size 40/0.04 taper, Group 3: apical preparation with M*two* Apical Files. All root canals were observed through scanning electron microscopy (SEM). Presence of superficial debris and smear layer was evaluated using a score system. Data were statistically analysed using the Kruskal-Wallis and Bonferroni tests with a level of significance set at $P < 0.05$.
Results: Kruskal-Wallis test revealed no differences among groups in the middle and coronal third ($P > 0.05$), while at the apical level, there was a significant difference for both residual debris and presence of smear layer between Group 1 and both Group 2 ($P = 0.003$ and $P = 0.014$) and 3 ($P = 0.012$ and $P = 0.021$), while no difference was present between Group 2 and Group 3 ($P = 0.871$ and $P = 0.923$).
Conclusions: Cleanliness of the apical third in terms of debris and smear layer was statistically better when an apical preparation was performed to a size 40/0.04 taper or with the use of the M*two* Apical Files.

Keywords: nickel-titanium alloy; root canal preparation; scanning electron microscopy.

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INTRODUCTION

Primary objective of root canal therapy is to thoroughly cleanse the root canal system, removing microorganisms and their substrates and organic and inorganic contents from the canal space [1]. Without proper chemomechanical instrumentation, the remaining irritants may reduce the success rate and cause failure of the treatment [2]. However, it remains one of the most difficult challenges in endodontic therapy [3]. Many studies have demonstrated that chemomechanical preparation of the root canal may result in a significant reduction of bacteria, but will not reproducibly leave bacteria-free root canals [4-6]. These objectives are more difficult to achieve in complex anatomical spaces, such as oval canals, in which it may be difficult to instrument the entire walls and not well cleaned recesses and infected dentine may remain after chemical and mechanical preparation [7].

The final apical preparation size further remains matter of debate [8]. To overcome the potential limits of instrumentation and irrigation in the apical area, enlargement of this area has been advocated for better cleansing [5,6,9-11]. In this regard, apical root-canal preparation by mechanical canal shaping has an antimicrobial effect via canal debridement [4,5,11-13]. Many studies have demonstrated that debris are more effectively removed when the apical preparation size is large [5,6,9,10,14]. Other studies have demonstrated that widely accepted endodontic cleaning and shaping techniques are inadequate [9,15,16]. This inadequate instrumentation could be attributed to the fact that root canal diameter is larger than the instrument caliber often used [17].

Recently, rotary files specifically designed for the apical preparation have been introduced on the market (M*two* Apical Files, Sweden & Martina, Padova, Italy; Hero Apical, Micro-Mega, Besancon, France). The M*two* endodontic instruments are a new type of NiTi rotary instruments for root canal preparation. The M*two* system has been completed with 3 rotary files specifically designed for the apical preparation, the M*two* Apical Files (A). The 3 apical files vary in tip size and taper. The innovative feature of these instruments is the high taper of the last apical millimeter. The A1 instrument presents a tip size (D0) of 0.2 mm and 15% taper in the first millimeter, thus measuring 0.35 mm in D1. A2 instrument presents a tip size (D0) of 0.25 mm and 15% taper in the first millimeter, thus measuring 0.4 mm in D1. A3 instrument presents a tip size (D0) of 0.25 mm and 20% taper in the first millimeter, thus measuring 0.45 mm in D1. The remaining portion of these instruments, from D1 to D16, present a 2% taper. To obtain this design, the apical millimeter of the instrument is not spiralized but it has two straight blades while maintaining a rounded non-cutting end. This design has been developed to obtain bigger preparation diameters in the apical portion of the root canals maintaining the anatomy of the apical foramen. The enhanced taper in the apical zone also provides resistance form against the condensation pressures of obturation and acts to prevent the extrusion of the filling material.

The aim of the present study was to compare the influence of type and dimensions of the apical preparation on the cleanliness of the apical area in molars by means of a scanning electron microscope analysis. The null hypothesis tested in the present study was that no difference in canal wall cleanliness exists between the different apical preparations used.

MATERIAL AND METHODS

Selection of samples

30 maxillary and 30 mandibular sound freshly extracted molars of similar length were selected for this study from a pool of extracted teeth. Mesio-buccal (MB) and disto-buccal (DB) root canals of the maxillary molars and the mesial root canals from the mandibular molars have been used. The teeth were stored in 0.1 thymol solution at room temperature and placed into 5.25% sodium hypochlorite solution for 20 minutes to remove the periodontal ligament. All remaining organic residues were removed from external root surfaces with a scaler, with careful examination under stereomicroscope at magnification x30 (Stemi SV6, Carl Zeiss S.p.A., Arese, Italy) to check for root fractures and to confirm that apex formation was complete. Roots with open apices, signs of apical root resorption and fracture lines were discarded.

The cusps were flattened and access to the pulp chamber was established with a cylindrical diamond bur (Komet # 6881, Komet-Brasseler, Lemgo, Germany) using a high-speed handpiece under copious water-cooling. The crowns were not removed at the level of the cementoenamel junction in order to preserve the normal trajectory of NiTi rotary instruments. After the root canal orifices were identified, patency of the MB and DB canals of the maxillary molars and MB and mesio-lingual (ML) canals of the mandibular molars was determined by using a size 10 K-Flexofile (Dentsply-Maillefer, Baillagues, Switzerland) to discard teeth with canal

obstructions. Two traditional analogic radiographs were taken in a bucco-lingual and mesio-distal direction for studying root canal anatomy and identify the radiographic apex. Teeth were fixed with wax on the X-ray film to maintain them perpendicular to the X-ray source and avoid movement during X-ray exposure. The radiographs were used to detect canals that joined each other. Only mesial roots of mandibular molars with two separate canals were included in the study. MB2 canals of maxillary molars were not used in the present study. If mesial root canals of mandibular molars were confluent they were discarded and replaced with specimens with separate root canals as confirmed radiographically. Only roots that demonstrated moderate curvatures (< 10°) [18] were selected for this study and roots with abrupt apical curvatures (with a radius of curvature ≤ 2 mm in the last 3 mm) were also excluded.

Root canal preparation

A single experienced operator (GP) prepared all root canals. Root canal working length was visually determined for each canal by inserting the size 10 K-Flexofile into each canal until the tip of the file became visible at the major foramen under 20X stereomicroscope and subtracting 0.5 mm from this measurement.

A total of 120 root canals were instrumented with M*two* NiTi rotary instruments in a simultaneous technique to a size 25/0.06 taper (instrumentation sequence tip size/taper: 10/0.04, 15/0.05, 20/0.06, 25/0.06) and were equally divided (10 MB and 10 DB root canals of maxillary molars and 10 MB and 10 ML root canals of mandibular molars) into three different experimental groups depending on the subsequently apical root canal preparation: Group 1: no further apical preparation was performed; Group 2: apical preparation with M*two* files using the instruments tip size 30/0.05 taper, size 35/.04 taper to a size 40/0.04 taper; Group 3: apical preparation with M*two* Apical Files using the instruments A1 and A2.

NiTi rotary instruments were powered at 280 rpm using a torque control motor with torque values already established for each M*two* instrument (Silver Motor, VDW, Munich, Germany). NiTi M*two* instruments tip size/taper 10/0.04, 15/0.05, 20/0.06, 25/0.06 were used in a simultaneous technique [19]. Instruments were each taken to working length with light apical pressure. As soon as the clinician felt a binding sensation, the instrument was withdrawn 1 - 2 mm so that it could be worked in a brushing action to selectively remove the interferences and to advance towards the apex. The instruments were used with lateral pressure in order to obtain a circumferential cut and allowed to rotate for few seconds after reaching the full length, before proceeding to the next size. M*two* instruments tip size 30/0.05 taper, size 35/0.04 taper to a size 40/0.04 taper and Apical Files A1 and A2 were only used in an up and down motion till the working length has been reached. The patency of the apical foramen was checked by passing the tip of a size 08 file through the foramen after each instrument of the M*two* sequence until completion of the root canal shaping. Each instrument was used to prepare maximum 5 root canals. Instruments with any sign of fracture or deformation have been discarded and replaced. Roots were embedded in polivinilsyloxane in order to not visualize the foramen during root canal instrumentation and to simulate presence of the surrounding tissues.

The same irrigation protocol was used for the different groups. All root canals were irrigated with 2.5 ml of 5.25% sodium hypochlorite (Niclor 5, Ogna, Muggiò, Italy) after each instrument and with 5 ml of 17% EDTA for 2 minutes after the last flush with sodium hypochlorite subsequently to the last instrument used at the end of the preparation. A final flush with 2.5 ml of sterile saline solution has been used to wash out all irrigant remnants. All irrigation procedures were performed by syringe and a 30-gauge needle (Navy Tip, Ultradent Products, South Jordan, USA). The needle was inserted 1 mm short of the binding point during instrumentation, and 1 mm short of the working length for the final irrigations after the preparation.

Scanning electron microscopy (SEM) preparation and analysis

All root canals were observed through scanning electron microscopy (SEM) to evaluate canal wall cleanliness in the coronal, middle and apical third, by measuring them after splitting before SEM analysis. The roots were split longitudinally as reported by Wu and Wesselink [9]. Two shallow longitudinal grooves were cut on each root in a bucco-lingual direction; care was taken that the grooves followed the curvature and did not penetrate into the canal. The roots were then split with a mallet and chisel made up of an adapted cementum spatula, resulting in a mesial and distal half of the root canal. Both halves were prepared for SEM investigation (Philips SEM 515, Eindhoven, the Netherlands). Representative photomicrographs were taken at different magnifications (x200, x1000).

A grading system was used to score the amount of

superficial debris and presence of smear layer according to the classification of Gutmann et al. [20], establishing a different score for the coronal, middle, and apical portions of the root canal of each section. The following criteria were used for debris evaluation (6 - 8 microscopic fields at magnification x200): 1 none to slight presence of superficial debris covering up to the 25% of the dentinal surface, 2 little to moderate presence of debris covering between 25% and 50% of the surface, 3 moderate to heavy presence of residual debris covering between 50% and 75% of the surface, 4 heavy amount of aggregated or scattered debris covering over 75% of the surface. The following criteria were used for smear layer evaluation (12 fields at magnification x1000): 1 little or no smear layer, covering less than 25% of the specimen; tubules visible and patent, 2 little to moderate or patchy amounts of smear layer, covering between 25% and 50% of the specimen; many tubules visible and patent, 3 moderate amounts of scattered or aggregated smear layer, covering between 50% and 75% of the specimen; minimal to no tubules visible or patent, 4 heavy smear layer covering over 75% of the specimen; no tubule orifices visible or patent.

The evaluations were carried out blindly by three operators who were unaware of the treatments that were rendered. Prior to scoring the test specimens, the examiners reviewed samples to ensure calibration and to reach a mutual understanding as to what amounts of superficial debris, smear layer, and patent or blocked dentinal tubules constituted each ranking from 1 to 4. Four photomicrographs of the superficial debris (x200) and four of the smeared layer (x1000) were taken to represent the four gradations of the scoring system. These photomicrographs served as visual reference standards for the examiners during the scoring of the test specimens. When different scores were attributed a discussion has been made between the evaluators to find an agreement.

Statistical analysis

Data were analysed using the Kruskal-Wallis test and Bonferroni multiple range test for multiple group comparisons. Statistical significance was considered at $P < 0.05$.

RESULTS

9 specimens (3 from Group 1, 2 from Group 2 and 4 from Group 3) were excluded from the study because they could not be evaluated due to damage occurring during sample preparation for SEM analysis.

The results of SEM analysis of the root canal walls concerning residual debris and smear layer are summarized in Table 1 and 2.

Kruskall-Wallis test revealed a statistical difference among groups in both residual debris and smear layer evaluation for what concern the apical third ($P = 0.0001$), while no differences among groups have been reported in the middle and coronal third ($P > 0.05$).

In terms of residual debris in the apical third, there was a significant difference between Group 1 and both Group 2 ($P = 0.003$, Bonferroni test) and 3 ($P = 0.012$, Bonferroni test), while no difference was present between Group 2 and Group 3 ($P = 0.871$, Bonferroni test).

Table 1. Number of specimens registered for each score in the different root canal thirds in the evaluation of residual superficial debris

	Score	Group 1 (n = 74)	Group 2 (n = 76)	Group 3 (n = 72)
Coronal third	**1**	64[a]	68[a]	64[a]
	2	8[a]	6[a]	6[a]
	3	4[a]	2[a]	1[a]
	4	0[a]	0[a]	0[a]
Middle third	**1**	50[a]	48[a]	44[a]
	2	12[a]	18[a]	14[a]
	3	10[a]	8[a]	10[a]
	4	2[a]	2[a]	4[a]
Apical third	**1**	4[a]	20[b]	16[b]
	2	24[a]	38[b]	36[b]
	3	36[a]	14[b]	16[b]
	4	10[a]	4[a]	4[a]

Note: Different superscript letters indicate statistical significant differences ($P < 0.05$) among groups using the Kruskal-Wallis test and Bonferroni multiple range test for multiple group comparisons.

Table 2. Number of specimens registered for each score in the different root canal thirds in the evaluation of residual smear layer

	Score	Group 1 (n=74)	Group 2 (n=76)	Group 3 (n=72)
Coronal third	**1**	60[a]	58[a]	58[a]
	2	6[a]	6[a]	8[a]
	3	4[a]	10[a]	2[a]
	4	4[a]	2[a]	4[a]
Middle third	**1**	52[a]	48[a]	48[a]
	2	8[a]	14[a]	8[a]
	3	6[a]	8[a]	12[a]
	4	8[a]	6[a]	4[a]
Apical third	**1**	8[a]	30[b]	30[b]
	2	42[a]	40[a]	36[a]
	3	20[a]	4[b]	4[b]
	4	4[a]	2[a]	2[a]

Note: Different superscript letters indicate statistical significant differences ($P < 0.05$) among groups using the Kruskal-Wallis test and Bonferroni multiple range test for multiple group comparisons.

In terms of presence of smear layer, there was a significant difference between Group 1 and Group 2 ($P = 0.014$, Bonferroni test) and Group 1 and Group 3 ($P = 0.021$, Bonferroni test). Differences between Group 2 and 3 were not significant ($P = 0.923$, Bonferroni test).

DISCUSSION

Thorough instrumentation of the apical region has long been considered to be an essential component in the cleaning and shaping process [8]. The last few millimeters that approach the apical foramen are critical in the instrumentation process [8,21], as it is the region that most likely harbours intraradicular bacteria associated with root canal treatment failure [22].

Mechanical instrumentation and irrigation are sound endodontic principles and essential components of successful endodontics. Research has shown that mechanical instrumentation greatly reduces the number of microorganisms remaining in the root canal system [4]. Mechanical instrumentation has been shown to reduce bacterial count even without irrigants or dressings [23]. However, irrigation with antimicrobial solutions is required to further reduce the number of microorganisms to clinically acceptable levels [4,10,24]. Furthermore, mechanical instrumentation with irrigation does not reliably disinfect an infected root canal system [5,6,25]. In this regard, apical root-canal preparation by mechanical canal shaping has been advocated to obtain an antimicrobial effect via canal debridement [5,6,9-11].

The results of the present study showed that a root canal preparation to a size 40/0.04 taper resulted in less residual debris and presence of smear layer in the apical third compared with the size 25/0.06 taper preparation, in agreement with other studies that have found better canal apical cleanliness with apical preparation [6,10,26]. When the apical preparation was performed with the M*two* Apical Files, no significant difference was found with the preparation to a size 40/0.04 taper. In the present study, no differences have been reported between the three groups in the middle and coronal thirds of the root canals. These results were expected because the preparation used in all the groups differed only in the apical third. In fact, after a common basic preparation to size 25/0.06 taper, the apical preparation to size 40/0.04 taper in Group 2 determined that the three instruments used (30/0.05, 35/0.04 and 40/0.04) touched only 8 mm of the root canal, reaching at this point a diameter of 0.72 mm versus 0.73 mm obtained initially by size 25/0.06 taper. The same concept is for group 3 in which the preparation with A1 and A2 Apical File touched only 4 mm of the root canal, reaching at this point a diameter of 0.46 mm versus 0.49 mm obtained initially by size 25/0.06 taper.

Furthermore, the results of the present study reported that a basic preparation to an instrument 25/0.06 taper is able to obtain a good cleanliness of the middle and apical third of root canals. This is probably due to the fact that, despite a complete contact between instruments and root canal walls is not possible, the dimensions of this basic preparation enlarge sufficiently the middle and coronal thirds to permit the irrigants to act well in these areas. Differently, in the apical third the differences between groups were statistically significant. In fact, the apical preparation

performed by two different approaches, revealed statistically cleaner root canal walls. It should be explained by the fact that debris created by root canal preparation may be more easily removed both mechanically and chemically when dimensions of the apical third have been enhanced, and that chelating agents may reach more efficiently the apex and may remove better the smear layer.

Explanation for these results may be also related to the benefits of using greater volume of irrigant, thus increasing the time of action during the preparation [27]. In fact, for Group 2 and 3, three and two instruments more than group 1 have been used respectively, so that three and two cycles of irrigation more have been performed respectively for Group 2 and 3. Furthermore, despite a flexible 30-gauge needle has been used in the present study as previously advocated to optimise the effectiveness of the irrigation [28,29], the diameter of the 30 gauge needles used in this study was 0.31 mm, exactly as the diameter obtained 1 mm short of the apex with the instrument 25/0.06. This correspondence in dimensions may have created more difficulties to the needle to reach the distance of 1 mm from the working length and irrigation may have been performed less efficiently than Group 2 and 3 in which at the end of the preparation a diameter of 0.44 mm and 0.4 mm have been reached in that point.

The observation that an enlarged apical preparation permits a better debridement of the apical third obtained in the present study corroborates results obtained by several studies [5,6,9,10]. Albrecht et al. [14] suggested that debris were more effectively removed using 0.04, 0.06, and 0.08 ProFile GT instruments when the apical preparation size was larger (size #40) compared with size #20 apical preparations. When a taper of 0.10 could be produced at the apical extent of the canal, there was no difference in debris removal between the two preparations sizes. All these articles report on the significance of apical third preparation for better cleaning and irrigation process, as underlined by van der Sluis et al. [30].

Clearly, a continuous taper 0.10 root canal preparation is often impossible to obtain, especially in small roots as used in the present study or in particularly curved root canals. In these situation, it seem useful the use of instruments like the M*two* apical files which maintain the apical foramen small (size #25) while enhancing dimensions of the apical millimetres through an exaggerate taper in the last millimetre of the instrument or to use for the apical preparation a sequence of instruments with progressively less taper (i.e. M*two* instruments tip size 30/0.05 taper, 35/0.04 and 40/0.04).

Despite apical preparation seems to be necessary as the most predictable way to clean and disinfect root canals to prevent apical periodontitis [31], especially in the most difficult anatomies [32], no study to date has shown a definitive relationship between apical preparation and clinical success or failure. Clinical seriously conducted prospective studies are mandatory to corroborate *in vitro* or *ex vivo* studies.

CONCLUSIONS

The null hypothesis has been rejected. In fact, the results of the present study showed that apical canal wall cleanliness was better when an apical preparation has been performed after a basic preparation of size 25/0.06 taper, irrespective of the technique used to perform the apical preparation.

No differences in terms of residual debris and smear layer evaluation have been reported among groups in the middle and coronal third of root canals.

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The authors deny any conflicts of interest.

The authors affirm that we have no financial affiliation (e.g., employment, direct payment, stock holdings, retainers, consultantships, patent licensing arrangements or honoraria), or involvement with any commercial organization with direct financial interest in the subject or materials discussed in this manuscript, nor have any such arrangements existed in the past three years. Any other potential conflict of interest is disclosed.

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