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Micro-CT Assessment of Filling Removal Effectiveness in the Apical Third of Curved Canals with Different Types of Anatomy

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Introduction: The aim of this study was to assess the effectiveness of filling removal material from the apical third of curved mesial root canals of mandibular molars. Reciprocating instrumentation followed by additional rotary instrumentation with instruments made of alloys with different heat treatments was evaluated. **Materials and Methods:** Thirty-six mesial roots of mandibular molars were divided into two groups: Group Class IV consisted of 16 roots with two independent canals, and Group Class II consisted of 20 roots with two canals that merged into one at their apical level. Each of these two groups were further divided into two subgroups, according to the additional rotary instrument used after the reciprocating instrumentation: Group RH and Group RM for Hyflex and Mtwo, respectively. After each procedural step, the roots were scanned by micro-tomography. After each step of filling removal, the Wilcoxon matched pair test and the Mann-Whitney test were used for the evaluation between groups. The significance level adopted was 5%. **Results:** Significant differences were observed between groups with different Class II and Class IV anatomies, regarding filling removal after Reciproc ($P<0.05$). After the use of an additional rotary instrumentation, no differences were observed between the two groups ($P>0.05$). **Conclusions:** In the apical third of mesial roots of mandibular molars with Class II anatomy, an additional rotary instrumentation was shown to be necessary for improving the removal of filling material after using the single-file reciprocating instrumentation technique.

Keywords: Curved Root Canals; Micro-computed Tomography; Root Canal Retreatment; Rotary Instrument

Introduction

Persistent apical periodontitis after primary endodontic treatment is mainly caused by bacteria that remain unaffected by the antimicrobial procedures [1]. When apical periodontitis persists after the root canal treatment, nonsurgical retreatment should be considered as the primary therapeutic approach. In the root canal retreatments, the filling material and cement must be removed from the canals as much as possible. An ideal filling should be easy to remove [2].

Nickel-titanium (NiTi) rotary and reciprocating systems have proved to be safer and more efficient than hand files for endodontic retreatment [3-6]. The thermomechanical treatment of NiTi files provides significant benefits to the efficacy and safety of endodontic instruments [7]. The M-Wire NiTi files contain austenite, R-phase and martensite in amounts that vary based on the different processing conditions, while the CM-Wire NiTi files are mainly in the martensitic phase with small amounts of R-phase [7-9]. Hence, conventional and M-wire NiTi files maintain their superelastic state, while CM-Wire does not have superelastic properties at body temperature. Furthermore, CM-Wire NiTi files that are in the martensitic phase in clinical conditions, retain the shape of the canal curvature during instrumentation of curved canals and do not straighten; therefore, the martensitic phase not only contributes to increasing instrument flexibility and resistance to fatigue, but also helps prevent procedural errors during instrumentation [10, 11]. Although increased flexibility could affect cutting

efficiency negatively, Hyflex CM–wire instruments (Coltene-Whaledent, Allstetten, Switzerland) have an enhanced cutting efficiency in lateral action when compared with conventional NiTi instruments [12]. Some studies have demonstrated that reciprocating NiTi instruments are safe and efficient in root canal retreatment procedures [4-6, 13] and rotary NiTi instruments are more effective than Hedstrom files in removing gutta-percha [14]. Others have shown that instrument hybridization is more effective for filling removal [15, 16].

The oval-shaped type of anatomy is prevalent in the mesial root canals of molars that merge into one in their apical third (Vertucci class II) [17]. The prevalence of this type of anatomy reached 54% of cases in some studies [18, 19]. In the present study, we observed a 52.5% prevalence of this anatomical type in the 40 mesial molar roots evaluated. The highly possible removal of filling material during endodontic retreatment must be achieved to promote the best level of disinfection [1]. This goal may be affected by the oval or flattened cross-section of these canals that merge into one in the apical part of the mandibular mesial roots, where the circular cross-section of rotary or reciprocating instruments presents greater difficulty in cleaning and disinfection [20]. Therefore, we also considered it relevant to evaluate whether there were differences regarding filling removal with Reciproc in different anatomies, in comparison with our previous study evaluating only one canal in mesial roots of mandibular molars [4]. In the previous study the percentage of filling removal with Reciproc was 93%.

The null hypotheses were that there would be no differences between groups regarding the effectiveness of filling removal from curved mesial canal roots of molars with two different types of anatomy, Vertucci class II and IV.

Materials and Methods

The present study was conducted after receiving the approval of the Research Ethics Committee of the Bauru School of Dentistry, University of São Paulo (CAAE: 44736215.9.0000.5417). The 40 human mandibular molars with curved mesial canals used in the study, were donated by the Human Tooth Bank of the University of São Paulo, São Paulo, SP, Brazil. The teeth were digitally radiographed in the buccolingual and mesiodistal directions, with a digital X-ray system (Schick CDR; Schick Technologies, Long Island, NY, USA), using an exposure time of 0.16 sec. This procedure was adopted with the purpose of selecting the teeth, to confirm full development and absence of internal root resorptions or calcifications. Forty teeth with mesial roots having canal curvatures between 25° and 35° were chosen. The root canal curvature angle was measured, as described by Schneider [21]. From this initial group, 36 teeth were selected and divided into two groups according to the Vertucci classification of anatomy [17]; Group Class IV consisted of 16 roots with independent canals, and Group Class II consisted of 20 roots with 2 canals that merged into one in the apical third. The groups were then further divided into two subgroups according to the instruments used in the root canal filling removal: Reciproc Plus Hyflex CM (Group RH) and Reciproc Plus Mtwo (Group RM). Group homogeneity in terms of anatomy, root canal length, curvature angle, and Vertucci classification [17] was confirmed and showed that the study groups were well balanced. The sample calculation was made by using the G*Power software (G*Power version 3.1 for Mac; Heinrich Heine, Universität Düsseldorf, Germany) and selecting the Wilcoxon-Mann-Whitney test of the t-test family. The alpha-type error of 0.05, a power of 0.95, and a ratio N2/N1 of 1 were also stipulated. A total of 8 samples per group were indicated as the ideal size required for no significant differences.

The tooth crowns were flattened with a diamond disc (FKG Dentaire, La Chaux-de-Fonds, Switzerland) to obtain a standardized specimen length of 16 mm. The working length was established by inserting a #10 K-file into each root canal until its tip was visible at the root apex, using an operating microscope (Alliance, São Paulo, SP, Brazil) at 8× magnification. The patency of the canals was confirmed by using a #10 K-file (Dentsply Maillefer, Ballaigues, Switzerland).

Endodontic treatment

PathFile instruments #13, #16, and #19 (Dentsply Maillefer) were used to create the glide paths; they were coupled to a VDW electric motor (VDW, Munich, Germany), operating at the speed of 300 rpm and torque of 60 g.cm. Subsequently, the teeth were instrumented with R25 Reciproc files (VDW, Munich, Germany) up to the working length, established at 1 mm short of the apex. The Reciproc instrument was used with the corresponding VDW electric motor in accordance with the Reciproc program, with three in-and-out pecking motions applied with light apical pressure. During glide path creation and root canal instrumentation, 2.5% sodium hypochlorite was used as irrigant solution. Final irrigation was performed with 1 mL of 17% ethylenediaminetetraacetic acid for 1 min, followed by a final rinse with 2 mL of 2.5% sodium hypochlorite dispensed with a 30-gauge needle (Navitips; Ultradent Products, South Jordan, UT, USA). The canals were then dried with R25 paper points; obturated with R25 cones (VDW), and AH-Plus sealer (Dentsply Maillefer), according to the single-cone technique. The teeth were again digitally radiographed, as

described above, to evaluate canal filling quality. The crowns were temporarily sealed with a provisional sealant (Citodur; Dorident, Vienna, Austria) and the specimens were stored at 37°C and 100% humidity for 3 months.

Initial removal of the filling material

The R25 Reciproc instrument was used for initial removal of the filling material from both groups, following the same protocol used in the original endodontic treatment, but a brushing motion was applied after each three in-and-out pecking motions. During the instrumentation steps and the final rinse, 2.5% sodium hypochlorite was used. The filling removal procedure was considered concluded when the canal walls were clean and smooth, and when no material was observed in the instrument flutes, when visualized under an operating microscope (Alliance, São Paulo, SP, Brazil).

Additional rotary instrumentation step

In Group RH, a HyFlex CM #40/0.04 instrument was used as an additional step for filling removal and re-instrumentation. In Group RM, an Mtwo #40/0.04 instrument was used as an equivalent additional procedure**.** After reaching the working length, both instruments were used with a brushing motion for 20 sec.

Micro-CT evaluation of the retreatment protocols

After completion of the storage period, the teeth were mounted on a custom attachment and scanned by a micro-computed tomography apparatus (SkyScan 1174; Bruker-microCT, Kontich, Belgium) using the following parameters: 50 kV, 800 µA, and isotropic voxel size of 39 µm. The other parameters used were: 360° rotation, 0.8° rotation steps and a 0.5 mm aluminum filter. Axial cross-sections were obtained with a beam hardening correction of 30% and a ring artefact correction of 4. Images of the specimens were reconstructed with NRecon software (NRecon version 1.6.3; Bruker-microCT, Kontich, Belgium) and the volumetric analyses of the 3D models were performed with similar parameters using CTAn software (CTAn version 1.12, Bruker-microCT, Kontich, Belgium). The models were divided into 3 segments of 3 mm each, which corresponded to the apical, middle and cervical portions of the root specimens. In this study, the apical portion of the mesial roots were evaluated. The filling material volume was calculated from the binarized area inside the region of interest, which included both root canals and isthmuses, using the same parameters. This final volume was recorded and converted into a percentage relative to the volume of the initial filling.

The micro-CT scans were repeated after each procedural step of the protocols tested in Groups RH and RM, using the same parameters.

Statistical analysis

Statistical analysis was performed using Prism 6.0 software (GraphPad Software Inc, La Jolla, CA, USA). The normality of the data was assessed by the Shapiro-Wilks test, and lack of normality led to the use of the Mann-Whitney test to compare the groups after each step of the retreatment protocols.

The Wilcoxon matched pair test was used to compare the reduction in filling material volume values obtained from performing each mechanical step of root canal instrumentation, after using the Reciproc R25 and after the additional step with HyFlex CM #40 and Mtwo #40 instruments. The Mann-Whitney test was used for the evaluation between groups. The significance level adopted was 5%.

Results

Filling removal

Class II and IV Groups after Reciproc

The filling material was not completely removed from inside the root canals. The remaining volume of filling material between the groups with different types of anatomy were significantly different. There were significant differences regarding filling removal after Reciproc between Group Class IV and Class II (P<0.05) (Table 1 and Figure 1).

Class II and IV Groups after using an additional rotary instrument, Hyflex (Group RH) and Mtwo (Group RM)

The filling material was not completely removed from inside the root canals in either group. The remaining volume of filling material after using Hyflex or Mtwo in the two groups did not differ significantly, independent of the anatomy (Table 1) (P>0.05).

In the intragroup evaluation, significant differences (P<0.05) were observed between each procedural step in terms of remaining filling material volumes. The use of Hyflex or Mtwo after Reciproc were both effective as an additional step regarding filling removal (Table 1 and Figure 1).

Table 1. Median, minimum and maximum values in percentage (%) reduction of filling material, after reciprocating, and after rotary instrumentation, respectively, in Group Class IV and Group Class II.

Group Class IV		Group Class II	
RH	**RM**	**RH**	**RM**
After Reciproc	**After Reciproc**	**After Reciproc**	**After Reciproc**
–80%^{aA} (38%–90%)	–85%^{aA} (29%–99%)	-60%^{bA} (35%-97%)	-56%^{bA} (39%-88%)
After Hyflex	**After Mtwo**	**After Hyflex**	**After Mtwo**
–85%^{aB} (56%–98%)	–93%^{aB} (70%–100%)	-87%^{aB} (55%-98%)	-76%^{aB} (65%-97%)

**Different superscript lower-case letters in each row indicate significant differences between groups (P<0.05); and different superscript capital letters in each column indicate significant differences within the same group after each procedural step (P<0.05)*

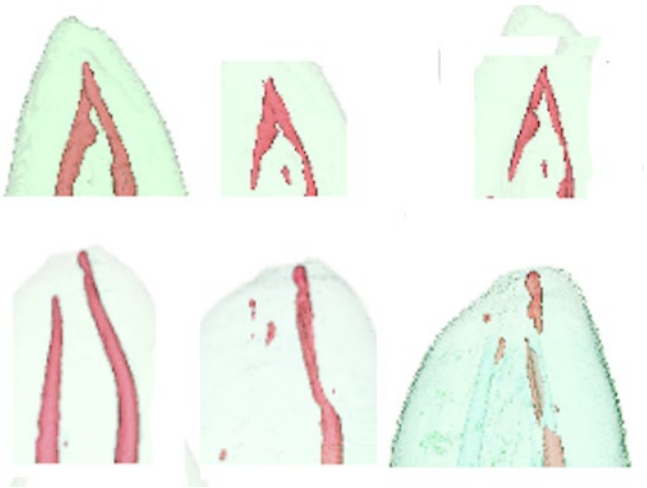

Figure 1. Micro-CT reconstructions of the apical third of two representative specimens from group RH with Vertucci class II *A*, *B* and *C*) Anatomy configuration; and class IV *D*, *E* and *F*); Before and after filling removal; *A* and *D*) Initial filling, *B* and *E*: after Reciproc; *C* and *F*) After Hyflex

Discussion

The null hypotheses were rejected since there were differences between groups regarding the efficacy of filling material removal from the apical third of curved mesial root canals of mandibular molars with different anatomies.

Mechanical techniques are widely adopted to remove the filling material, due to the greater ability of NiTi instruments in remaining centered in the canal, and in reducing the working time [22].

The instruments in the two evaluated protocols were unable to completely remove the filling material from the apical third of root canals, which was in agreement with the results of previous studies [23-25]

A high incidence of ramifications in the apical third of the root canal system, as well as the presence of isthmuses with an apical diameter measuring approximately 0.350 mm, have been found in micro-CT studies [17, 18]. According to these findings, the Reciproc R25 instrument with a 0.25 tip would probably be ineffective in cleaning this area. Furthermore, we also evaluated two types of NiTi alloys with different surface and heat treatments: the conventional alloy of the Mtwo instrument, and an alloy known as "controlled memory" (CM) of the HyFlex instrument, with alleged properties of greater flexibility and fatigue resistance [7, 10]. Another study demonstrated that the HyFlex CM instrument preserved the original root canal shape better than the WaveOne and Reciproc reciprocating instruments, due to its greater flexibility [26].

In our study, there were no significant differences between HyFlex CM (Control memory NiTi) and Mtwo instruments (conventional NiTi) in regard to filling removal.

The areas left untouched by reciprocating and rotary instruments could range from 10% to 50% of the root canal walls [27, 28]. The rotary instruments Hyflex CM and Mtwo used in this study had an equivalent 0.40 tip that could have improved the filling removal after the use of Reciproc R25 with a 0.25 tip. A study showed that a HyFlex CM 40, 0.04 instrument was effective and safer to use than a Reciproc 40, 0.04 instrument considering the shaping ability of the apical third of mesial root canals of mandibular molars [26].

A NiTi instrument should have high resistance to fracture and fast cutting action [12, 27]. In some studies, the control memory NiTi instruments have shown higher resistance to fracture than conventional NiTi instruments, without loss of the dentin cutting and filling removal efficiency [7, 11]. Therefore, the control memory NiTi instruments could be considered as a good choice for an additional step in the procedure of filling removal from curved mesial root canals of mandibular molars, as observed within the limits of this study.

The Reciproc instruments in the two protocols evaluated were unable to completely remove the filling material from the root canals in their apical third, which is in agreement with the results of previous studies [4, 29], with worse results for the roots in which the canals merged into one. A micro-CT study showed findings of a high incidence of ramifications in this portion of the root canal system, as well as the presence of isthmuses with an apical diameter measuring approximately 0.350 mm [18, 19]. As a result, the Reciproc R25 instrument with a .25 tip would probably be ineffective in cleaning this area.

In a clinical setting, according to the results of this study, special attention must be given to the anatomy of the canals in the mesial root of mandibular molars. The poorer filling removal in canals with Vertucci Class II configuration showed that the approach of performing complementary steps, such as additional rotary instrumentation, to enlarge the apical third could be useful. Some studies have shown that hybridization of reciprocating, rotary and manual instruments leads to more effective protocols for filling removal [15, 16, 25], with supplementary steps such as agitating the irrigant solution by means of passive irrigant agitation (PUI), reciprocating plastic instruments and NiTi finishing instruments [25, 30-32]. The action of irrigating substances is improved with the enlargement of the apical region, confirming the results of a previous study [33]. This enlargement is even more important in oval-shaped canals, where cleaning and removal of filling material are more difficult to achieve [23].

Studies about cleaning and filling removal have been conducted in single canals [6, 13], possibly because they are more predictable. In our previous study, in which mesial roots of molars with two canals were evaluated, the isthmus area was not considered [4].

In the present study, the less effective filling removal in Group Class II with canals that merged in their apical third (Vertucci class II) was statically significant when compared with Group IV with two independent canals (Vertucci class IV). These findings suggested that additional and supplementary approaches should be considered useful for enhancing filling removal and cleaning in the apical third of mesial root canals of mandibular molars, particularly when they merge into one in their apical third.

Conclusion

None of the protocols were capable of completely removing the filling material from the curved root canals used in this study. Regarding the use of reciprocating instrumentation in different types of anatomy, IReciproc instruments showed to be more effective in filling removal in curved mesial canals of mandibular roots with two independent canals and independent foramens better than in the canals that merged into one foramen in their apical third. The use of an additional rotary instrumentation step after the initial filling removal procedure using reciprocating instrumentation, increased the amount of removed filling material. The greater flexibility of NiTi control memory instruments than that of the conventional NiTi instruments did not reduce the effectiveness of filling removal.

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Conflict of interest
None.

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Authors' contributions
Lincoln de Campos Fruchi, main author, conception, writer, design, materials, data collection, analyses and interpretation, literature review. Murilo Priori Alacalde, second author, design and critical review. Pablo Amoroso-Silva: design and critical review. Clovis Monteiro Bramante: critical review. Rodrigo Ricci Vivan: critical review. Marco Antônio Hunger Duarte: Supervision, critical review, Analysis and interpretation

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