Abstract


Aim To compare the cleaning effectiveness of manual, hybrid and rotary instrumentation techniques in primary molar teeth.

Methodology Fifteen primary molars were selected. After endodontic access, the teeth were immersed in a medium containing Enterococcus faecalis and divided into three groups, according to the root canal instrumentation technique: group 1 – manual, group 2 – hybrid and group 3 – nickel–titanium (NiTi) rotary files. For microbiological evaluation, comparisons before and after instrumentation were performed using the paired Student’s t-test. One-way ANOVA complemented with the Student’s t-test was used to compare the percentage of microbial reduction. Instrumentation time was evaluated by Kruskal–Wallis and Student–Newman–Keuls tests. Images obtained under scanning electron microscopy were analysed by three blinded examiners, and kappa statistics was used to evaluate calibration among examiners. The most frequent results among examiners were analysed using Kruskal–Wallis and Student–Newman–Keuls tests.

Results The hybrid technique required a significantly longer instrumentation time than the manual and rotary techniques (P < 0.05). All techniques tested were able to significantly reduce the number of E. faecalis (P < 0.05). The hybrid technique was associated with the highest intracanal bacterial reduction, with a statistically significant difference compared with manual instrumentation (P = 0.01). Manual instrumentation resulted in the lowest amount of debris and the highest amount of smear layer when compared with the rotary and hybrid techniques (P < 0.05). There was no difference between rotary and hybrid instrumentation in the removal of debris and smear layer.

Conclusion The use of NiTi rotary files is an option for root canal instrumentation in primary teeth.

Keywords: Enterococcus faecalis, instrumentation, primary tooth, root canal preparation.

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Introduction

The premature loss of primary teeth may cause changes in the chronology and sequence of eruption of permanent teeth. Maintenance of primary teeth until physiological exfoliation contributes to mastication, phonation and aesthetics and prevents deleterious habits in children (Bodur et al. 2008). Therefore, primary teeth with pulpitis or necrosis are indicated for endodontic treatment (Leonardo et al. 2008).

The outcome of endodontic treatment in primary teeth is dependent on microbial reduction as a result of chemomechanical preparation (Jha et al. 2006), removal of residual pulp tissue and debris, and maintenance of the original canal curvature during
instrumentation. Anaerobic microorganisms are present in most of the microbiota of primary teeth with necrotic pulps, and aerobic microorganisms are present in 60% of the cases (Garcez et al. 2006). Streptococcus and Enterococcus faecalis are an important cause of endodontic treatment failure, particularly because of microbial resistance after conventional treatment (da Silva et al. 2006). The presence of accessory foramina in the furcation and ectopic root resorption makes cleaning and shaping of primary root canals more difficult (Pinheiro et al. 2009).

Chemomechanical preparation for disinfecting root canals in primary teeth may be performed by a manual technique using K-files or a rotary system with nickel–titanium (NiTi) files (Barr et al. 2000). Although manual instrumentation is widely used in primary teeth, there are limitations regarding effective cleaning of root canals, possible ledge formation, perforations, dentine compaction and instrument fracture (Silva et al. 2004).

Rotary instrumentation in primary teeth has several drawbacks, such as the high cost of NiTi instruments, the need to discard the files regularly and the need for operator training (Barr et al. 1999, 2000, Crespo et al. 2008). Conversely, rotary systems require shorter instrumentation time than manual techniques. This factor is relevant in paediatric dentistry because it allows faster procedures while maintaining quality and safety, in addition to reducing fatigue of the patient and the professional (Kummer et al. 2008).

There are few clinical case reports (Barr et al. 2000, Silva et al. 2004) and laboratory studies (Nagaratna et al. 2006) using rotary systems for root canal preparation in primary teeth, but there are several studies on permanent molars. Considering the anatomical, histological and chemical differences between permanent and primary dentitions, such as increased mineralization of permanent teeth and morphological changes owing to the presence of physiological or pathological root resorption, results obtained in permanent teeth cannot be transposed to primary teeth (Kummer et al. 2008, Pinheiro et al. 2009). Siqueira et al. (2002) reported a reduction in E. faecalis after rotary instrumentation in permanent teeth, but there are no studies assessing intracanal bacterial reduction in deciduous teeth.

The aim of this study was to compare the cleaning capacity and instrumentation time of manual, hybrid and rotary techniques in primary teeth.

Materials and methods

Tooth selection

This study was approved by the Research Ethics Committee of the Unicastelo institution and conducted in accordance with the provisions of the Declaration of Helsinki. The results from a pilot procedure were used to calculate the sample size. Calculation was performed using the two-sample paired t-test in the BioStat 4.0 software (two related samples, mean difference in log 10 colony-forming units before and after instrumentation, alpha 0.01). Five teeth per group were required to obtain statistical power. Therefore, 15 primary molars were selected from the Human Tooth Bank of the Children’s Clinic at the School of Dentistry, Pontifícia Universidade Católica (PUC) – Campinas, Brazil (Table 1). The inclusion criteria were as follows:

- primary molars with at least 11.0 ± 1.0 mm of working length;
- absence of external and/or internal pathological root resorption;
- absence of perforation in the internal and/or external furcation area;
- moderate root angulation (Schneider 1971).

Tooth preparation

The teeth were washed under running water, immersed in 2.0% chlorhexidine (Fórmula & Ação, São Paulo, SP, Brazil) for 24 h and individually embedded in acrylic resin (VIPI, Pirassununga, São Paulo, Brazil) up to the cementoenamel junction. The teeth were radiographed with X-ray film (Kodak, São José dos Campos, SP, Brazil) using radiographic equipment (ProDental, Lisbon, Portugal) with an exposure time of 0.8 s for the purpose of planning access to the root canals and analysing root canal anatomy.

Endodontic access was obtained with a sterile, size 6 spherical carbide bur (KG Sorensen, São Paulo, SP, Brazil), and after locating the canals, access was completed with a size 3082 diamond bur (KG Sorensen). The working length was determined visually. Root canal length was established by inserting a size 10 hand K-file (Dentsply Maillefer, Ballaigues, Switzerland) into the canal until the file tip could be seen at the apical foramen. A silicone stop was placed in the cusp corresponding to the root canal, the file was withdrawn and the working length was determined by subtracting 1 mm from the canal length (Bernardes et al. 2007).
The teeth were then sterilized in an autoclave (Brasdermica, São Paulo, SP, Brazil).

Standard strains of *E. faecalis* ATCC 19433 (LabCenter, Campinas, SP, Brazil) were inoculated into 200 mL of brain heart infusion (BHI) broth (LabCenter) for 5 days at 37°C in an atmosphere of 85% nitrogen (N2), 10% carbon dioxide (CO2) and 5% hydrogen (H2), achieved by using the anaerobic atmosphere-generating envelope system (Oxoid Ltd., Basingstoke, UK). The teeth were immersed in BHI containing a standard strain of *E. faecalis* (0.5 McFarland scale). For bacterial sampling, a sterile paper point (Dentsply Maillefer) of an anatomical diameter compatible with that of the canal was introduced into the root canal, retained in position for 30 s and immediately transferred to the BHI medium.

The teeth were randomly divided into three groups (each tooth was considered as an experimental unit):

- **Group 1 (n = 5)** – manual instrumentation: root canals were prepared manually using size 15, 20 and 25 Kerr K-type files (Dentsply Maillefer) to the working length (Yared et al. 2002, Silva et al. 2004).
- **Group 2 (n = 5)** – hybrid instrumentation with the ProTaper system and K-files (Dentsply Maillefer): root canals were prepared initially by manual instrumentation using a size 15 K-file, followed by S1 and S2 of the rotary system, manual instrumentation with size 15 and 20 K-files, rotary system F1, manual instrumentation with size 25 K-file and finally rotary system F2.
- **Group 3 (n = 5)** – rotary instrumentation with the ProTaper system (Dentsply Maillefer): root canals were prepared using the rotary system in the following sequence: S1 and S2 followed by F1 and F2.

In all groups, curved root canals were prepared in accordance with the anticurvature filing method proposed by Abou Rass et al. (1980). Rotary instruments (groups 2 and 3) were driven by an electric micromotor (X-Smart Dentsply Maillefer, Ballaigues, Switzerland).

The following irrigants were used during instrumentation in all groups: Endo-PTC (urea peroxide + Tween 80 + Carbowax; Fórmula & Ação) and 0.5% sodium hypochlorite (Dakin’s solution; Fórmula & Ação). Before instrumentation, 100 mg of Endo-PTC was placed in the pulp chamber. The root canals were irrigated with 1 mL of 0.5% sodium hypochlorite at each change of instrument. The time spent on irrigation was included in the total instrumentation time.

A stopwatch (Tecnbras, São Paulo, SP, Brazil) was used to record instrumentation time for each group. At the end of instrumentation, a second bacterial sample was collected from primary teeth using a sterile paper point (Dentsply Maillefer) of an anatomical diameter compatible with that of the canal, which was introduced into the root canal, retained in position for 30 s and immediately transferred to the BHI medium. All samples were diluted to $10^{-3}$ and seeded on blood agar plates (LabCenter) to count the number of viable bacteria.

### Homogenization and dilution

The samples were homogenized in a tube agitator for 1 min, and three decimal dilutions were made in 4.5 mL of peptone water. Of the decimal dilutions, three 25-μL aliquots were seeded using the micropipette technique on the surface of blood agar plates (Le Goff et al. 1997).

### Culture conditions

After seeding, the cultures were incubated at 37 °C for 5 days in an atmosphere of 85% N₂, 10% CO₂ and 5% H₂ atmosphere, obtained using the anaerobic atmosphere-generating envelope system. After this period, the total number of colony-forming units per millilitre (CFU mL⁻¹) of viable bacteria was visually assessed.

### Scanning electron microscopy analysis

Longitudinal grooves were made with a diamond disc (KG Sorensen) on the mesial and distal aspects of each tooth to guide the section with forceps of samples for

<p>| Table 1 Distribution of deciduous molars among the three experimental groups |
|---------------------------------|---------------------------------|---------------------------------|
| MT, manual technique; HT, hybrid technique; RT, rotary technique; UM, upper deciduous molars; LM, lower deciduous molars; MB, mesiobuccal root; P, palatal root; DB, distobuccal root; ML, mesiolingual root; DL, distolingual root; D, distal root. |</p>
<table>
<thead>
<tr>
<th>Deciduous molars (n)</th>
<th>MT</th>
<th>HT</th>
<th>RT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Root canals (n)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MB (5); P (2)</td>
<td>UM (2); LM (3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DB (3); ML (3)</td>
<td>MB (5); P (2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DL (1); D (1)</td>
<td>DB (3); ML (2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total number of root canals (n)</td>
<td>15</td>
<td>15</td>
<td>15</td>
</tr>
</tbody>
</table>
scanning electron microscopy (SEM) analysis. The areas with the best visibility of the apical root canal were evaluated (Soukos et al. 2006). The samples were dehydrated in alcohol, placed on metal stubs and coated with gold for observation under SEM (JEOL 5200; Jeol, Tokyo, Japan) at 100, 500 and 1000× magnification.

Cleaning capacity was defined as the ability to remove microorganisms, debris and smear layer from the root canal. Dentine chips, pulp remnants, larger particles and aggregates appearing haphazardly on the root canal walls were classified as debris. A surface film consisting of remnants of dentine and pulp tissues, with a smeared structured appearance, was defined as smear layer. All images were assessed by three examiners who were blinded to which group each sample belonged and also to the absence, partial or complete presence of debris and/or smear layer. The examiners assigned a score of 1–3 to each image with regard to smear layer and debris, as follows (Zmener et al. 2005):

Scores for debris:
1. No debris or isolated small particles (±40 μm) were present.
2. Debris covered more than 50% of the canal walls.
3. Debris almost entirely covered the canal walls.

Scores for smear layer:
1. All dentinal tubules were open, and no smear layer was present.
2. Some dentinal tubules were open, and the rest were covered by a smear layer.
3. Continuous smear layer covered the canal walls, and no dentinal tubules were seen.

Statistical analysis
For microbiological evaluation, the results (in CFU mL⁻¹) were subjected to descriptive analysis. Within-group comparisons before and after instrumentation were performed using the paired Student’s t-test. One-way analysis of variance (ANOVA) complemented with the Student’s t-test was used to compare the percentage of microbial reduction between groups. Kruskal–Wallis and Student–Newman–Keuls tests were used to comparatively evaluate instrumentation time between groups.

Kappa statistics was used to evaluate the level of calibration among examiners. The most frequent results among examiners were analysed using Kruskal–Wallis and Student–Newman–Keuls tests. The Biostat version 4.0 was used for data analysis. Significance was set at P < 0.05 for all analyses.

Results
The hybrid technique required significantly longer instrumentation time than the manual and rotary systems (P < 0.05) (Table 2). All techniques tested were able to significantly reduce the number of E. faecalis (P < 0.05). The hybrid technique was associated with the highest intracanal bacterial reduction, with a statistically significant difference compared with manual instrumentation (P = 0.01) (Table 3).

Interexaminer calibration is shown in Table 4. Manual instrumentation was associated with the lowest amount of debris and the highest amount of smear layer when compared with the rotary and hybrid techniques (P < 0.05). There was no difference between rotary and hybrid instrumentation in the degree of debris and smear layer (Table 5) (Figs 1–3).

Discussion
Enterococcus faecalis is a Gram-positive anaerobic bacterium that is often associated with persistent endodontic infections in primary teeth (Jett et al. 1994). Because E. faecalis is the microorganism most commonly associated with endodontic treatment failure (Zehnder & Guggenheim 2009), a standard strain of this bacterium was chosen in the present study. The extraction of E. faecalis with paper points followed the method used by Pinheiro et al. (2009), Siqueira et al. (1999, 2002).

The association of Endo-PTC and 0.5% sodium hypochlorite has been found to promote satisfactory cleaning of root canals compared with other solutions (Yamazaki et al. 2010). Pinheiro et al. (2009) observed

| Instrumentation time (minutes) using manual, hybrid and rotary techniques in primary molars

<table>
<thead>
<tr>
<th>Instrumentation time</th>
<th>Median ± interquartile</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manual technique (MT) n = 5</td>
<td>12.00 ± 0.00</td>
<td>MT × HT = 0.03</td>
</tr>
<tr>
<td>Hybrid technique (HT) n = 5</td>
<td>18.00 ± 1.00b</td>
<td>MT × RT = 0.25</td>
</tr>
<tr>
<td>Rotary technique (RT) n = 5</td>
<td>10.00 ± 2.00</td>
<td>HT × RT = 0.001</td>
</tr>
</tbody>
</table>

*Kruskal–Wallis and Student–Newman–Keuls tests were used to compare instrumentation time between different techniques.

bSignificantly different from groups MT and HT; HT and RT.
clinically that manual instrumentation using Endo-PTC and 0.5% sodium hypochlorite resulted in a reduction of 82.59% of viable bacteria in primary molars with necrotic pulps. For this reason, and also to obtain results close to those that may be achieved in the clinical practice of paediatric dentistry, the present study used this irrigant solution during instrumentation for all three techniques tested. Therefore, differences in the reduction in *E. faecalis*, smear layer and debris are associated with the different instrumentation techniques, as cleaning resulting from Endo-PTC with 0.5% sodium hypochlorite was the same for all three groups.

The results revealed no significant difference in cleaning effectiveness between manual and rotary techniques, which is in agreement with other studies (Silva *et al.* 2004, Kleier & Averbach 2006, Nagaratna *et al.* 2006). Rotary instrumentation required a shorter preparation time, a finding consistent with other reports (Silva *et al.* 2004, Nagaratna *et al.* 2006, Kummer *et al.* 2008, Cheung & Liu 2009).

To assess the potential advantages of combining manual and rotary techniques, the present study

<p>| Table 3 | Colony-forming units (CFU) per millilitre (10³) and percentage of reduction in <em>Enterococcus faecalis</em> before and after instrumentation using manual, hybrid and rotary techniques in primary molars* |</p>
<table>
<thead>
<tr>
<th>CFU mL⁻¹ (10³)</th>
<th>Before</th>
<th>After</th>
<th>P-value</th>
<th>Percentage of reduction</th>
<th>Mean ± SDb</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>MT (n = 5)</td>
<td>21.03 ± 15.02</td>
<td>0.65 ± 0.68c</td>
<td>0.03</td>
<td>96.90 ± 1.30d</td>
<td>MT × HT = 0.01</td>
<td></td>
</tr>
<tr>
<td>HT (n = 5)</td>
<td>23.99 ± 16.38</td>
<td>0.10 ± 0.10e</td>
<td>0.03</td>
<td>99.58 ± 0.62d</td>
<td>MT × RT = 0.12</td>
<td></td>
</tr>
<tr>
<td>RT (n = 5)</td>
<td>15.94 ± 4.56</td>
<td>0.21 ± 0.16f</td>
<td>0.001</td>
<td>98.88 ± 1.08d</td>
<td>HT × RT = 0.26</td>
<td></td>
</tr>
</tbody>
</table>

*Paired Student’s t-test was used to compare CFU mL⁻¹ before and after instrumentation using different techniques.

One-way ANOVA and Student’s t-test were used to compare the percentage of reduction between different techniques.

Significantly different before and after instrumentation.

Significantly different from MT and HT.

MT, manual technique; HT, hybrid technique; RT, rotary technique; SD, standard deviation.

<table>
<thead>
<tr>
<th>Table 4</th>
<th>Evaluation among examiners (kappa statistics)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kappa</td>
<td>Debris</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>P-value</td>
<td>0.762</td>
</tr>
<tr>
<td>Confidence interval (95%)</td>
<td>Upper: 1.0</td>
</tr>
<tr>
<td>Lower: 0.523</td>
<td>Lower: 0.63</td>
</tr>
</tbody>
</table>

<p>| Table 5 | Percentage of scores assigned by three examiners to the amount of debris and smear layer after instrumentation using manual, hybrid and rotary techniques* |</p>
<table>
<thead>
<tr>
<th>Scores for debris</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>MT</td>
<td>83.33</td>
<td>16.66</td>
<td>0</td>
<td>MT × HT = 0.05</td>
</tr>
<tr>
<td>HT</td>
<td>16.66</td>
<td>83.33</td>
<td>0</td>
<td>MT × RT = 0.01</td>
</tr>
<tr>
<td>RT</td>
<td>0</td>
<td>100</td>
<td>0</td>
<td>HT × RT = 0.62</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Scores for smear layer</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>MT</td>
<td>16.66</td>
<td>66.66</td>
<td>16.66</td>
<td>MT × HT = 0.02</td>
</tr>
<tr>
<td>HT</td>
<td>0</td>
<td>100</td>
<td>0</td>
<td>MT × RT = 0.03</td>
</tr>
<tr>
<td>RT</td>
<td>16.66</td>
<td>66.66</td>
<td>16.66</td>
<td>HT × RT = 0.89</td>
</tr>
</tbody>
</table>

Significantly different from MT and HT; MT and RT.

*Kruskal–Wallis and Student–Newman–Keuls tests were used for statistical analysis.

MT, manual technique; HT, hybrid technique; RT, rotary technique.
evaluated the hybrid instrumentation technique, alternating the use of stainless steel and NiTi files. The hybrid technique promoted the greatest reduction in E. faecalis, but also required the longest instrumentation time. The greater reduction in E. faecalis obtained with the hybrid technique may be associated with the simultaneous action of manual and rotary instruments. Probably, one explanation for the present results, once there is no available literature of hybrid instrumentation technique in primary teeth (Madan et al. 2011), is that the preparation of the coronal third by the use of rotary system S1 and S2 enabled the cleaning of both middle and apical thirds by manual instrumentation. Root canals were finally shaped using F1, then manual instrumentation with size 25 K-file and finally rotary system F2. Therefore, the hybrid technique used a larger number of instruments than any other technique.

For all techniques, non-debrided sites with remaining debris were found, as observed in most studies related to root canal cleaning (Ahlquist et al. 2001, Schäfer & Schlingemann 2003). The hybrid and rotary techniques were not significantly different regarding the removal of debris and smear layer, a finding consistent with previous studies (Schäfer & Schlingemann 2003). Manual instrumentation had the lowest amount of debris and the largest amount of smear layer compared with the rotary and hybrid techniques.

Conclusion

In the present study, the hybrid technique was associated with the greatest reduction in E. faecalis, but required longer instrumentation time. The rotary and manual techniques had a similar ability to reduce E. faecalis, but the rotary system required shorter instrumentation time and produced less smear layer. The use of NiTi files is an option for root canal instrumentation in primary teeth.

Conflict of interest and source of funding

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References


