SEM evaluation of root canal dentin morphology after Ni-Ti instrumentation

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ABSTRACT: Purpose: The aim of this study was to compare, through scanning electron microscope (SEM), the ability of four Ni-Ti rotary instrument systems in shaping root canal walls and their ability in removing smear layer and dentin debris. Methods: Forty-six extracted single-rooted human teeth were divided into four groups and prepared to size 35 (Alpha System, FlexMaster, MFile) or 30 (NRT files). Irrigation was carried out with NaOCl and EDTA. Three parameters were evaluated in the coronal, middle, and apical thirds of the root canals: smear layer morphology, pulpal-inorganic debris presence and surface profile morphology. Data were statistically analyzed using the Kruskal-Wallis test (ANOVA). Results: None of the Ni-Ti rotary instrument systems resulted in being able to obtain constantly regular shaped surfaces in apical thirds, where smear layer, pulpal and inorganic debris were often present. Conclusions: NRT file specimens resulted in being relatively free from debris and smear layer and gained better results and scores at any canal level (Journal of Applied Biomaterials & Biomechanics 2009; 7: 116-22). Key words: Debris, Ni-Ti, Rotary instruments, Scanning electron microscope, Smear layer

INTRODUCTION

The main objective of biomechanical instrumentation is the total elimination of micro-organisms from the infected pulp tissue and debris from the root canal (1, 2). It is important to develop an endodontic technique that produces a well-tapered root canal with a minimal amount of smear layer and debris (3). The smear layer is removed because of its potential negative effects (4), as it may harbor micro-organisms (5), prevent antimicrobial agents from gaining access to the infected dentin tubules (1, 2, 5) and compromise adaptation of sealing materials to the root canal walls (6, 7). Consequently, the ability of many Ni-Ti instruments to remove debris and smear layer has been tested (8, 9, 10, 11). A scanning electron microscope (SEM) investigation suggested that the design features of the cutting blade of Ni-Ti instruments may influence cleansing efficiency (12).

FlexMaster (VDW, Munich, Germany) Ni-Ti files are among the most comprehensively investigated rotary instruments (13). The cutting blades have no radial lands to provide effective removal of dentin. The cross-sectional profile is convex so that the alloy core is exposed to less stress during torsional loading.

MFile (Komet/Gebr. Brasseler, Lemgo, Germany) instruments have been introduced into clinical practice. They are indicated for the treatment of straight or curved canals with a Schneider angle of less than 30 degrees, but not for canals with an elevated angle of curvature or calcified canals. Only preliminary research on the shaping ability of MFile is currently available (14).

Alpha System (Komet/Gebr. Brasseler, Lemgo, Germany) instruments are designed to shape canals with narrow, medium or wide diameters, since apical ISO sizes from 20 to 35 and tapers from 0.10 to 0.02 are available. The entire system is composed of 11 instruments. Their Ni-Ti core is covered by a titanium nitride coating the manufacturer claims will prevent premature blunting of the blades. All instruments are manufactured with non-cutting tips. The canal is prepared with the crown-down technique.

NRT files (Mani, Inc, Tochigi, Japan) are designed specifically for the crown-down technique. The files are manufactured with a modified rectangular shaped core. This shape is considered safer by limiting the occurrence of file self-threading.

The aim of this study was to evaluate the ultrastructural morphology of root dentin canal walls of extracted teeth prepared with different Ni-Ti rotary techniques. The parameters used to evaluate cleaning efficacy were the amount and morphology of the smear layer, the presence of pulpal and dentinal debris, and the morphology of the dentin surface profile.
MATERIALS AND METHODS

Selection of samples

Forty-six single-rooted, human maxillary incisors, mandibular premolars and canines of similar length extracted for periodontal reasons were selected. All teeth were stored at 4 °C in saline solution immediately after extraction. The crown of each tooth was removed at the level of the cementum enamel junction (CEJ) so that root segments of approximately 15 mm in length could be obtained. Two longitudinal grooves were prepared on the palatal/lingual and buccal surfaces of each root using a diamond bur in a high-speed water-cooled handpiece in order to facilitate vertical splitting with a chisel after canal instrumentation.

The patency of apical foramina was standardized by inserting a size 08 K-file (Dentsply-Maillefer, Ballaigues, Switzerland) so that the tip was just visible. Individual working length (WL) was calculated 0.5 mm short of this position. Teeth with apical diameters larger than size 15 were excluded from the study. Finally, samples were placed in small blocks of polyvinylsiloxane (Platinum 95, Zetalabor, Zhermark, Rovigo, Italy) to avoid irrigation fluid flushing out of the foramen.

Canal preparation

Specimens were randomly divided into four groups, which were instrumented using a crown-down preparation with different Ni-Ti rotary systems (Fig. 1): 12 with Alpha System (Komet/Gebr. Brasseler, Lemgo, Germany), 12 with FlexMaster (VDW, Munchen, Germany), 12 with MFile (Komet/Gebr. Brasseler, Lemgo, Germany) and 10 with NRT files (Mani, Inc, Tochigi, Japan).

Irrigation was performed at any instrument change, with a total amount of 7.5 ml of 5% NaOCl (Niclor 5, Ogna, Muggio, Italy) and 7.5 ml of 10% EDTA (Tubuliclear, Ogna, Muggio, Italy) solutions, equally shared according to the number of instruments employed. EDTA was always placed and kept in canals before using any Ni-Ti system. The irrigation regime was carried out with a 27 gauge diameter endodontic needle (Molteni Jet APS, Molteni Dental, Scandicci, Italy) inserted into the root canal to the deepest level possible.

Each specimen was previously instrumented at the coronal third with Glidden Gates burs sizes 4, 3, 2. Each sequence was used following a crown-down rotary mechanical technique with a low speed (300 rpm for FlexMaster; 400 rpm for Alpha System-MFile-NRT files) endodontic handpiece (Tecnika Digital Torque Control Motor; ATR, Pistoia, Italy). Instruments were used for no more than five different canal preparations and then discarded. Apical preparation was set to size 15 using hand instruments K-file 10 and 15. One trained operator performed all root canal instrumentations. After canal preparation in each group, a final 1 mL aliquot of 10% EDTA solution was left in situ for 3 min, followed by a 1 mL 5% NaOCl aliquot for 3 min.

Instrumentation techniques

The sequence used in this study corresponded to the manufacturers’ instructions for each group of rotary Ni-Ti instruments (Tab. I). With all teeth used, the described uniform protocol was followed. After any change of instrument canal patency was always verified with a size 15 K-file. After completion of the instrumentation and irrigation, each canal was dried with sterile paper points.

Scanning electron microscope preparation

Immediately after canal preparation, each sample was split into two halves. The section including most of the apex was fixed in 4% glutaraldehyde in 0.2 M sodium cacodylate buffer solution at 4 °C. The sample was then dehydrated in graded concentration alcohol, dried using a critical point drier (E 3000, Polaron, Thermo VG Scientific, East Grinstead, West Sussex, UK), gold sputtered (Sputter Coater; SPI, Toronto, Canada) and observed using an SEM (JEOL 5200; JEOL, Tokyo, Japan). Images were saved in digital format using specific

Fig. 1 - Micrograph showing instruments shapes at x150. a: Alpha System (size 25, taper 0.06); b: FlexMaster (size, 25 taper 0.04); c: MFile (size 30, taper 0.04); d: Mani NRT (size 25, taper 0.04).
software (SEMsafore; JEOL) and scored by two trained operators in a double-blind manner: a consensus was reached between the two examiners regarding any discrepancies.

**Scoring system**

Three different areas (coronal, middle, and apical third) were analyzed by comparing the dentin surface morphology. To score all areas of each specimen, a predefined scale was used to evaluate three different parameters: smear layer, pulpal and inorganic debris and dentin surface profile and divided into four different levels (Alfa, Bravo, Charlie and Delta) (Tab. II). Alfa and Bravo levels were considered clinically acceptable endodontic preparations, whereas Charlie and Delta represented unacceptable clinical preparations. Data were statistically analyzed using the Kruskal-Wallis test (ANOVA).

**TABLE I - TOTAL NUMBER OF INSTRUMENTS USED, SEQUENCE AND WORKING LENGTH (WL)**

<table>
<thead>
<tr>
<th>System</th>
<th>FlexMaster (eight instruments)</th>
<th>MFile (four instruments)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sequence</td>
<td>Intro file (coronal third)</td>
<td>6% taper, size 30 (coronal third) (WL – 6 mm)</td>
</tr>
<tr>
<td></td>
<td>6% taper, size 25</td>
<td>4% taper, size 30 (middle third) (WL – 3 mm)</td>
</tr>
<tr>
<td></td>
<td>6% taper, size 20</td>
<td>2% taper, size 30 (apical third) (WL)</td>
</tr>
<tr>
<td></td>
<td>4% taper, size 25</td>
<td>2% taper, size 35 (apical third) (WL)</td>
</tr>
<tr>
<td></td>
<td>4% taper, size 30 (middle third)</td>
<td>2% taper, size 35 (apical third) (WL)</td>
</tr>
<tr>
<td></td>
<td>2% taper, size 25</td>
<td>2% taper, size 30 (apical third) (WL)</td>
</tr>
<tr>
<td></td>
<td>2% taper, size 30</td>
<td>2% taper, size 35 (apical third) (WL)</td>
</tr>
<tr>
<td></td>
<td>2% taper, size 35 (apical third)</td>
<td>2% taper, size 35 (apical third) (WL)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>System</th>
<th>Alpha System (five instruments)</th>
<th>NRT files (eight instruments)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sequence</td>
<td>10% taper, size 45 (coronal third)</td>
<td>6% taper, size 30</td>
</tr>
<tr>
<td></td>
<td>6% taper, size 30 (coronal-middle third)</td>
<td>4% taper, size 30</td>
</tr>
<tr>
<td></td>
<td>4% taper, size 30 (middle third)</td>
<td>6% taper, size 25</td>
</tr>
<tr>
<td></td>
<td>2% taper, size 30 (apical third)</td>
<td>4% taper, size 25 (WL)</td>
</tr>
<tr>
<td></td>
<td>2% taper, size 35 (apical third)</td>
<td>6% taper, size 25 (WL)</td>
</tr>
</tbody>
</table>

**TABLE II - SCALE OF VALUES ASSIGNED FOR THE THREE DIFFERENT PARAMETERS EVALUATED**

<table>
<thead>
<tr>
<th></th>
<th>Alfa</th>
<th>Bravo</th>
<th>Charlie</th>
<th>Delta</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smear layer</td>
<td>Absent, more than 75% of tubules exposed and completely free from smear layer. Tubules open</td>
<td>Present in limited areas, less than 75% of tubules uncovered. Tubules partially open</td>
<td>Present, tubules visible in limited areas and partially closed. Less than 50% of dentinal tubules visible</td>
<td>Homogeneous smear layer above all present dentin. Dentinal tubules not visible</td>
</tr>
<tr>
<td>Pulpal and Inorganic debris</td>
<td>Completely absent</td>
<td>Minimal presence</td>
<td>Presence of connective pulpal debris and/or inorganic debris covering a small portion of dentin surface</td>
<td>Significant presence of inorganic debris everywhere and/or an organized collagenous matrix covering dentin surface</td>
</tr>
<tr>
<td>Dentin surface profile</td>
<td>Absence of irregularities and grooves. Smooth surface</td>
<td>Isolated irregularities and grooves. Isolated islands of predentin</td>
<td>Partially irregular, with limited not-instrumented area of predentin</td>
<td>Irregular with grooves, depression and area of not-instrumented dentin where predentin was visible</td>
</tr>
</tbody>
</table>

**RESULTS**

No statistically significant differences (p>0.01) were found at the apical third for any parameter considered, regardless of the rotary system employed. At the apical level the highest scores were collected for each variable (smear layer, debris and surface profile) in all specimens analyzed (Tab. IIIa).

Considering medium thirds (Tab. IIIb), collected values were significantly different for debris and surface profile parameters (p<0.01). Instrument comparison disclosed that achieved variances were significant only between FlexMaster and NRT files for debris, and between MFile and NRT files for surface profile regularity (p<0.01).

At the coronal level (Tab. IIIc), results obtained with each group of instruments had no statistical value for smear layer or debris variables; the only noticeable mismatches concerned the surface profile obtained with MFile.
and NRT files (p<0.01), and Alpha System compared with NRT files (p<0.01).

Statistical analysis of all rotary systems at the coronal third showed statistical relief with reference to the surface profile variable, but not to smear layer or debris parameters. Results were rounded up or down to the first decimal place.

**DISCUSSION**

Several studies have demonstrated that SEM is a suitable method for the comparison of dentin root surface morphology after preparation using different instruments (3, 8, 9, 11, 12, 15-19). Nevertheless, evaluation of the amount of smear layer and debris is subjective and may provide incomplete information.

This study showed a substantial portion of dentin surface free from smear layer that was only present in the apical third (Fig. 2). A few samples of different groups presented a thick multilayered smear layer at the apical level with a typical “tree bark” configuration (20). Many researchers have demonstrated that larger apical preparations increase canal cleanliness because of the increased volumes of irrigant and deeper needle penetration (21-23). All samples in this study were prepared up to size 35 diameter, which appears to be the minimum instrumentation size needed for penetration of irrigants into the apical third of the root canal (24). The combination of NaOCl and EDTA was probably responsible for the removal of the

**TABLE IIIa - VALUES OF THREE PARAMETERS DIVIDED IN ALPHA-BRAVO (ACCEPTABLE) AND CHARLIE-DELTA (NOT ACCEPTABLE) BETWEEN THE FOUR GROUPS OF INSTRUMENTS AT APICAL THIRD. RESULTS WERE ROUNDED UP OR DOWN TO THE FIRST DECIMAL PLACE. NO STATISTICALLY SIGNIFICANT DIFFERENCES (P>0.01) WERE FOUND BETWEEN ANY PARAMETER CONSIDERED**

<table>
<thead>
<tr>
<th>Apical Third</th>
<th>Smear Layer %</th>
<th>Debris %</th>
<th>Surface Profile %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Alfa</td>
<td>Bravo</td>
<td>Charlie</td>
</tr>
<tr>
<td>ALPHA SYSTEM</td>
<td>8.3</td>
<td>25</td>
<td>41.7</td>
</tr>
<tr>
<td>FLEXMASTER</td>
<td>0</td>
<td>66.7</td>
<td>25</td>
</tr>
<tr>
<td>MFİLE</td>
<td>0</td>
<td>25</td>
<td>58.3</td>
</tr>
<tr>
<td>NRT FILES</td>
<td>10</td>
<td>20</td>
<td>60</td>
</tr>
</tbody>
</table>

Results were rounded up or down to the first decimal place. No statistically significant differences (p>0.01) were found between any parameter considered.

**TABLE IIIb - VALUES OF THREE PARAMETERS DIVIDED IN ALPHA-BRAVO (ACCEPTABLE) AND CHARLIE-DELTA (NOT ACCEPTABLE) BETWEEN THE FOUR GROUPS OF INSTRUMENTS AT MEDIUM THIRD. RESULTS WERE ROUNDED UP OR DOWN TO THE FIRST DECIMAL PLACE. COLLECTED VALUES WERE SIGNIFICANTLY DIFFERENT FOR DEBRIS AND SURFACE PROFILE PARAMETERS (P<0.01)**

<table>
<thead>
<tr>
<th>Medium Third</th>
<th>Smear Layer %</th>
<th>Debris %</th>
<th>Surface Profile %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Alfa</td>
<td>Bravo</td>
<td>Charlie</td>
</tr>
<tr>
<td>ALPHA SYSTEM</td>
<td>33.3</td>
<td>25</td>
<td>16.7</td>
</tr>
<tr>
<td>FLEXMASTER</td>
<td>16.7</td>
<td>58.3</td>
<td>16.7</td>
</tr>
<tr>
<td>MFİLE</td>
<td>16.7</td>
<td>41.7</td>
<td>41.7</td>
</tr>
<tr>
<td>NRT FILES</td>
<td>50</td>
<td>50</td>
<td>0</td>
</tr>
</tbody>
</table>

Results were rounded up or down to the first decimal place. Collected values were significantly different for debris and surface profile parameters (p<0.01)

**TABLE IIIc - VALUES OF THREE PARAMETERS DIVIDED IN ALPHA-BRAVO (ACCEPTABLE) AND CHARLIE-DELTA (NOT ACCEPTABLE) BETWEEN THE FOUR GROUPS OF INSTRUMENTS AT CORONAL THIRD. RESULTS WERE ROUNDED UP OR DOWN TO THE FIRST DECIMAL PLACE**

<table>
<thead>
<tr>
<th>Coronal Third</th>
<th>Smear Layer %</th>
<th>Debris %</th>
<th>Surface Profile %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Alfa</td>
<td>Bravo</td>
<td>Charlie</td>
</tr>
<tr>
<td>ALPHA SYSTEM</td>
<td>66.7</td>
<td>33.3</td>
<td>0</td>
</tr>
<tr>
<td>FLEXMASTER</td>
<td>41.7</td>
<td>41.7</td>
<td>16.7</td>
</tr>
<tr>
<td>MFİLE</td>
<td>50</td>
<td>50</td>
<td>0</td>
</tr>
<tr>
<td>NRT FILES</td>
<td>90</td>
<td>10</td>
<td>0</td>
</tr>
</tbody>
</table>

Results were rounded up or down to the first decimal place. No statistical value for smear layer or debris variables were found; the only noticeable mismatches concerned the surface profile obtained with MFİLE and NRT files (p<0.01), and the Alpha System compared with NRT files (p<0.01)
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smear layer and much of the circumferential dentin collagen and mineralized dentin wall from the most superficial part of the tubules, in agreement with recent observations on the clinical implications of the smear layer (5, 25, 26).

Areas free from smear layer presented many lateral branches grouped around tubule orifices, a typical effect of NaOCl action (27). As shown by the small orifices of lateral branches (Fig. 3) left by the removal of collagen fibrils, NaOCl irrigation probably removes them, masking the dentin surface. It is interesting that despite differences in instrument design and operative procedures all systems produced a dentin surface with a similar morphology in the medium (Fig. 4) and coronal (Fig. 5) thirds. It is possible that the role of the instrument is affected by the high efficacy of the irrigant solutions used in this study.

This study showed that FlexMaster produced more samples with a reduced amount of smear layer and a better surface profile, but the specimens instrumented with this system collected the highest scores for debris parameter. The Alpha System failed to instrument canal walls effectively in the coronal and apical thirds and completely remove the smear layer created from the dentinal surfaces, especially along the middle and apical thirds. Despite this, the Alpha System thoroughly removed debris at the coronal and especially at the middle levels.

MFile removed debris especially at the apical thirds,
where it proved the most effective rotary instrument. Despite unacceptable percentage values obtained for each variable in the coronal thirds, after instrument-
tation with the MFile system we observed the largest smear layer and the worst surface profile in the middle and apical thirds.

NRT files achieved excellent results in the coro-
nal and middle thirds for every parameter considered. Instead, apical thirds shaped with NRT files showed similar smear layer and debris build-ups to those ob-
served in roots instrumented with the other rotary files, but smoother and more regular dentinal surfaces. Our in vitro SEM analysis showed that NRT files were the most effective rotary system in preparing root canal dentin.

NaOCl and EDTA irrigants probably had a major ef-
fect on the results by helping to remove smear layer and de-
bris. The irrigants also improved instrument longevity, lowering friction between dentin and NiTi cutting edges.

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