SEM evaluation of canal wall dentine following use of M*two* and ProTaper NiTi rotary instruments

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Abstract

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Aim To compare using scanning electron microscopy (SEM) root canal walls following instrumentation *in vitro* with two different rotary NiTi instruments. The hypothesis was that no difference should be observable between the experimental groups in terms of debris on canal walls and surface morphology.

Methodology Twenty-four single-rooted human teeth were selected. Two types of NiTi instruments were used, M*two* (Sweden & Martina, Padova, Italy) and ProTaper (Dentsply Maillefer, Ballaigues, Switzerland). Irrigation for both groups was performed after each instrument change with 5% NaOCl, 3% H_2O_2 and 17% EDTA solutions. Three different areas (coronal, middle and apical thirds) of the root canal were evaluated using SEM. The canal wall of each sample was assessed and compared using a predefined scale of four parameters, namely, smear layer, pulpal debris, inorganic dentine debris, surface profile. Data were analysed statistically using the Kruskal–Wallis test (ANOVA).

Results A statistically significant difference ($P < 0.01$) was found between the apical third and the middle and coronal thirds for both groups. No difference was observable between instrumentation groups. In the apical third canal walls were often contaminated by inorganic debris and by smear layer. In the apical third, the surface profile was affected by uninstrumented regions, comprising dentine depressions and grooves in which predentine was still visible.

Conclusion Both instruments produced a clean and debris-free dentine surfaces in the coronal and middle thirds, but were unable to produce a dentine surfaces free from smear layer and debris in the apical third. The presence of deep grooves and depression on dentine walls in the apical third may well explain the presence of less-instrumented areas.

Keywords: dentine, NiTi rotary instruments, root canal preparation, SEM, smear layer.

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Introduction

All endodontic instruments create dentine debris and a smear layer as a consequence of their action on root canal walls (Torabinejad *et al.* 2002, Jeon *et al.* 2003). This debris may be compacted along the entire surface of canal walls increasing the risk for bacteria 'contamination' and reducing the adaptation of sealer and gutta-percha (Bowman & Baumgartner 2002). Furthermore, this debris may be compacted apically and create an apical plug that prevents the complete filling of this important region (Iqbal *et al.* 2003).

It is also important that endodontic instruments remove dentine and pulpal debris from the entire root canal wall and create a canal free from bacteria.

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Nickel-titanium (NiTi) instruments represent a relatively new approach to the rapid preparation of canals with standardized taper (Thompson & Dummer 2000, Bergmans *et al.* 2001, 2002, Peters *et al.* 2003). The amount, the thickness and the type of smear layer produced by new NiTi instruments must be assessed (Torabinejad *et al.* 2002). It is also important to determine the presence of grooves and concave areas where debris may be packed and bacteria trapped. These areas may be responsible for the presence of uninstrumented dentine along the entire length of root canal and may result in inadequate adaptation of the filling material (Bowman & Baumgartner 2002).

Recently, new rotary NiTi instruments with different configuration and design have been marketed as M*two* (Sweden & Martina, Padova, Italy) and ProTaper (Dentsply Maillefer, Ballaigues, Switzerland). Both instruments present positive rake angles, no radial lands, progressive blade camber (pitch) in the apical–coronal direction and a noncutting tip (Berutti *et al.* 2003, Blum *et al.* 2003). The M*two* cross-sectional design resembles that of the S-file (Dobo-Nagy *et al.* 2002).

The ProTaper cross-sectional design resembles that of a reamer, with three machined cutting edges and convex core. The ability of rotary instruments to remove dentine and pulpal debris during shaping is obviously connected to the flute and cross-sectional design (Gambarini & Laszkiewicz 2002).

Scanning electron microscopic (SEM) analysis appears to be an adequate method to investigate the influence of endodontic instruments on the morphology of dentine surfaces, and has been well described (Ahlquist *et al.* 2001).

The aim of this study was to evaluate the ultrastructural morphology of root canal walls of extracted teeth prepared using two different rotary NiTi systems. The amount and the morphology of smear layer, the presence of pulpal and dentinal debris and the morphology of inner dentine walls were parameters for the evaluation of shaping and cleaning efficacy.

Materials and methods

Selection of samples

A total of 24 single-rooted, human maxillary single-canalled incisors, mandibular premolars and mandibular canines of similar length and extracted for periodontal reasons, were selected. The crown of each tooth was removed at the level of the cementum enamel junction (CEJ) in order to obtain root segments of approximately 12 mm in length. Two longitudinal grooves were prepared on the palatal/lingual and buccal surfaces of each root with a diamond bur used with a high-speed water-cooled handpiece to facilitate vertical splitting with a chisel after canal instrumentation. Teeth were randomly numbered and divided into two groups.

Patency of the apical foramina was standardized by inserting a size 15 K-file (FKG, La Chaux-de-Fonds, 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. Teeth with sclerotic canals or with an altered apex were not included.

Canal preparation

Samples were prepared with two different instruments: group M ($n = 12$) samples with M*two* NiTi instruments (Sweden & Martina) (Fig. 1); group P ($n = 12$) samples with ProTaper NiTi instruments (Dentsply Maillefer) (Fig. 2).

Canals of both groups were irrigated with 1 mL of 5% NaOCl (Niclor 5; Ogna, Muggiò, Italy) followed by 1 mL of 3% H_2O_2 solutions (Ogna) and followed by 0.5 mL of 10% EDTA (Tubuliclean) after each change of instrument. The method was previously described by Mayer *et al.* (2002) and Niu *et al.* (2002). After canal preparation a final 1 mL aliquot of 17% EDTA solution was left *in situ* for 2 min and replaced by 1 mL of 5% NaOCl for 3 min. Each sample was then irrigated with tap water before SEM processing. All irrigation procedures were delivered with a 27-gauge needle (Molteni, Firenze, Italy). A 20 : 1 reduction handpiece (W & H Dentalwerk Bürmoos GmbH, Bürmoos, Austria) powered with a torque-controlled electric stepper motor (Tecnika Digital Torque Control Motor; ATR s.r.l., Pistoia, Italy) were employed for both instrumentation groups with a consistent rotation of 300 r.p.m.

Mtwo

The sequence used corresponded to the manufacturer's instructions. Five instruments were used: M*two* 10/.04; M*two* 15/.05; M*two* 20/.06; M*two* 25/.06; M*two* 30/.05. Initial negotiation of root canal space was performed using a size 15 manual K-file (FKG) used in a watch-winding motion to assure the presence of a glide path (Blum *et al.* 2003) as far as the foramen.

M*two* system requires the introduction of each instrument directly to WL, maintaining permanent

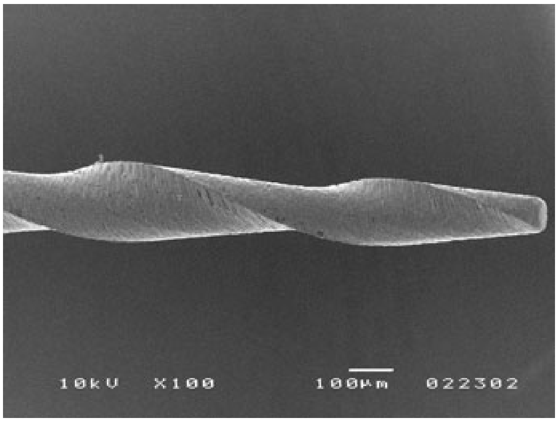

Figure 1 Micrograph demonstrating the 'S-file' aspect of M*two* (size 10 taper .04) instrument with two positive rake angles cutting edges and noncutting tip.

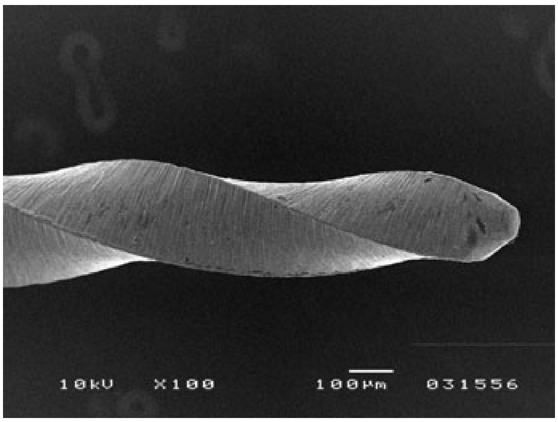

Figure 2 Micrograph showing F1 ProTaper (size 20, progressive taper) instrument with three positive rake angles cutting edges and noncutting tip.

rotation (300 r.p.m.), with slight in-and-out movement and whilst gradually forcing apically in the following sequence:

1. size 10 .04 taper instrument used at WL;
2. size 15 .05 taper instrument used at WL;
3. size 20 .06 taper instrument used at WL;
4. size 25 .06 taper instrument used at WL;
5. size 30 .05 taper instrument used at WL.

After complete instrumentation and irrigation, each canal was dried with sterile paper points.

ProTaper

The sequence used in this study was proposed by the Endodontic Unit of Bologna University for slightly curved canals (curvature gauge <5°) and are partially in agreement with a previous report (Blum *et al.* 2003). After patency verification with a size 15 manual K-file (FKG), the instruments were used in a crown-down fashion:

1. SX ProTaper at two-third of WL;
2. S1 at WL – 1 mm;
3. F1 at WL;
4. F2 at WL;
5. F3 at WL.

All instruments were inserted into root canal in a continuous in-and-out movement; they were never forced apically. After the complete instrumentation and irrigation, each canal was dried with sterile paper points.

SEM preparation

Immediately after canal preparation each sample was split into two halves with a stainless steel chisel. The section with the most visible part of the apex was conserved and fixed in 4% glutaraldehyde in 0.2 M sodium cacodylate buffer solution at 4 °C, dehydrated in graded concentration alcohol, dried with a critical point drier (E 3000; Polaron, West Sussex, UK) and then gold sputtered (Sputter Coater; SPI, Toronto, Canada) and observed with SEM (JEOL 5200; JEOL, Tokyo, Japan). After a general survey of the canal wall from the apex to the most coronal part, six SEM photomicrographs were obtained at a standard magnification of 2000× at each third (coronal, middle and apical). Specific areas of dentine were observed at greater magnification (5000×, 10 000× and 15 000×).

The images were saved digitally with specific software (SemAfore; JEOL) and scored in a double-blind manner by two trained operators.

Scoring system

The absence or presence of *smear layer*, *pulpal debris*, and *inorganic debris* were rated and scored on four appearances using a predefined scale and selected SEM pictures (Prati *et al.* 2004). To assess the dentine *surface profile* the presence of grooves, pits and predentine areas was evaluated (Table 1).

Statistical analysis

Data were plotted in the Statgraphics**plus* (Manugistics, Rockville, MD, USA) program and analysed with the Kruskal–Wallis test (ANOVA). For each single

Table 1 Scale of values assigned to the four different parameters evaluated

	1	2	3	4
Smear layer	Absent, more than 75% of tubules exposed and free from smear layer	Present in limited areas, less than 75% of tubules uncovered	Present, tubules visible in limited areas and partially closed	Homogeneous smear layer present above all dentine
	Tubules completely opened	Tubules partially opened	Less than 50% of dentinal tubules visible	Dentinal tubules not visible
Pulpal debris	Absent	Minimal presence of pulpal-fibrous debris	Partial presence of pulpal-fibrous debris	Presence of an organized collagenous matrix
Inorganic debris	Absent	Minimal presence	Often present	Present everywhere and covering dentine surface
Surface profile	Absence of irregularities	Isolated irregularities and grooves	Partially irregular, with limited not-instrumented areas	Irregular with grooves, areas of uninstrumented dentine

parameter box and whisker plots, with lateral notch for 95% confidence, were drawn showing the difference between the two instruments and between the canal thirds.

Results

Box and whisker plots (Figs 3–6) show the results for each parameter amongst the two groups and the three canal areas (coronal, middle and apical thirds). Completely clean root canals were not found with any of the instruments. The coronal thirds were cleaner and relatively free from debris.

Smear layer

Smear layer was present mainly in the apical third. In the coronal and middle thirds smear layer was not present and dentinal tubules were clearly visible and free from smear plugs (Fig. 7). The average diameter of dentine tubules was 2–2.5 μm.

The presence of smear layer was significantly greater in the middle and apical thirds in comparison with the coronal third. Representative micrographs show the presence of smear layer and its morphology in the apical thirds of canals shaped with M*two* and ProTaper instruments (Figs 8 and 9).

Several areas of dentine showed peritubular and intertubular erosion (Fig. 10). Dentinal tubules appeared larger and with a funnel entrance. In the middle thirds, excessive erosion often led to the conjunction of two or more tubular orifices.

Only the apical thirds showed isles of smear layer, which had been burnished and partially compressed. Smear plugs were observed in the perpendicular view of several samples.

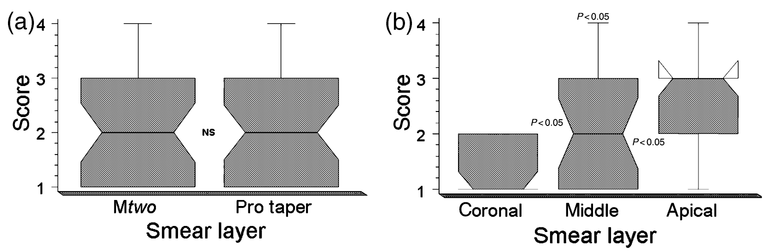

Figure 3 Box and whisker plots showing: (a) no statistical difference between groups M and P regarding the production of smear layer; (b) an increasing amount of smear layer from coronal to apical thirds.

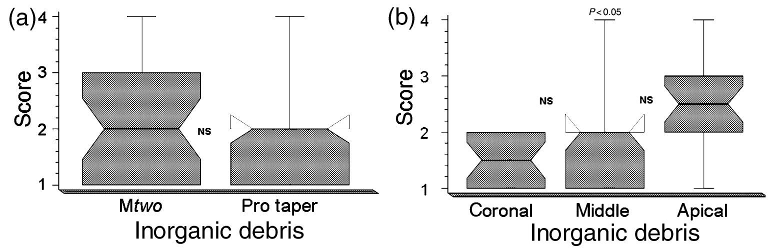

Figure 4 Box and whisker plots showing: (a) no statistical difference between groups M and P with respect to the presence of inorganic debris; (b) significantly higher amount of inorganic debris at apical third compared with coronal third.

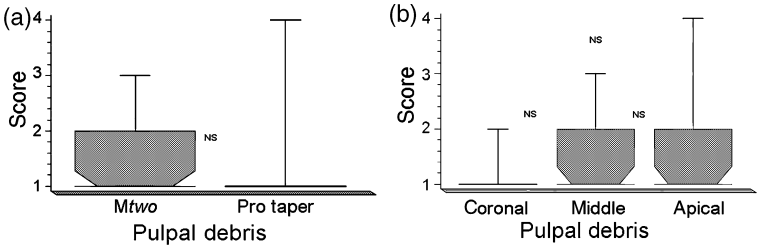

Figure 5 Box and whisker plots showing: (a) no statistical difference between groups M and P relating to the presence of pulpal debris; (b) pulpal debris absence at coronal third, their presence was limited at middle and apical thirds, however no significant difference was found.

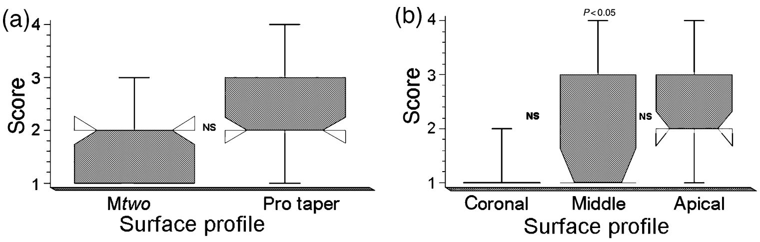

Figure 6 Box and whisker plots showing: (a) no statistical difference between group M and P regarding the surface profile parameter; (b) significantly less homogenous surface profile at apical third compared with coronal third.

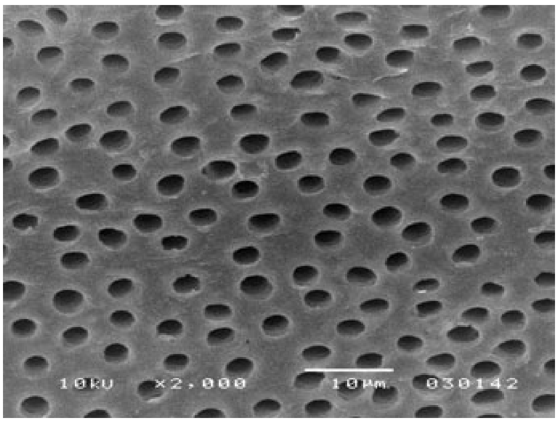

Figure 7 Micrograph demonstrating the absence of smear in coronal third and visible tubular orifices (group P).

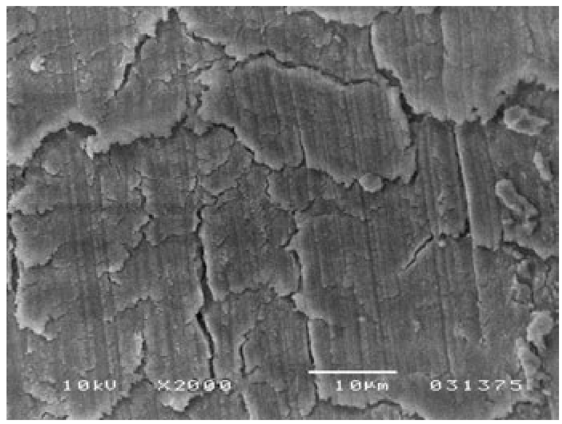

Figure 8 A compacted smear layer was present at apical third in group P. Dentinal tubules were not observable and patent.

Inorganic debris

Inorganic debris was easily discerned from pulpal debris and detected only in the apical third of some samples. Dimensions of dentine debris ranged from 1.5 to 20–30 μm (Fig. 11). As demonstrated by box and whisker plot (Fig. 2) the score obtained by the two instruments was similar.

Pulpal debris

The amount of pulpal debris were limited in all thirds (Fig. 5). Occasionally, in some samples, a collagenous matrix spread along root canal walls was observed in apical third. In these cases it was difficult to observe the morphology of dentine as it was masked by pulpal debris (Fig. 12).

Surface profile

The surface profile of canal walls was homogeneous both in the coronal and middle thirds. No pits, grooves or other superficial irregularities were detected in these areas. Predentine areas were observed only in several limited uninstrumented areas in the apical thirds of both groups. Predentine was observed only in grooves and depression of canal walls (Fig. 13).

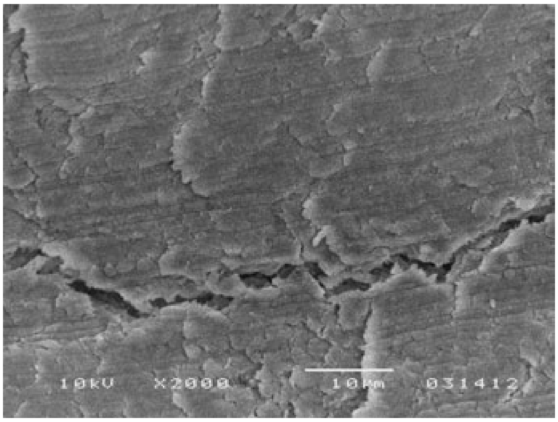

Figure 9 Groups P and M showed a similar thick smear layer in the apical third.

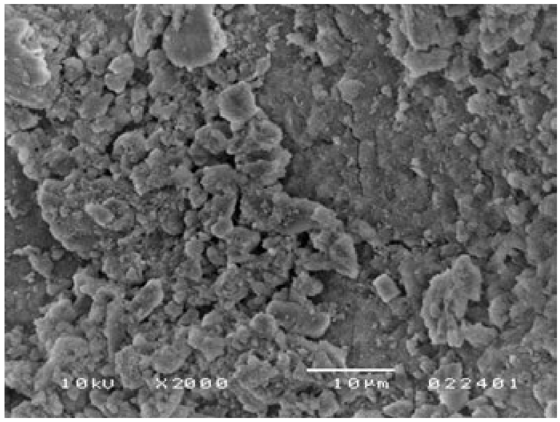

Figure 11 Dentine inorganic debris was detected in the apical third. Dimensions ranged from 1.5 to 30 μm (group P).

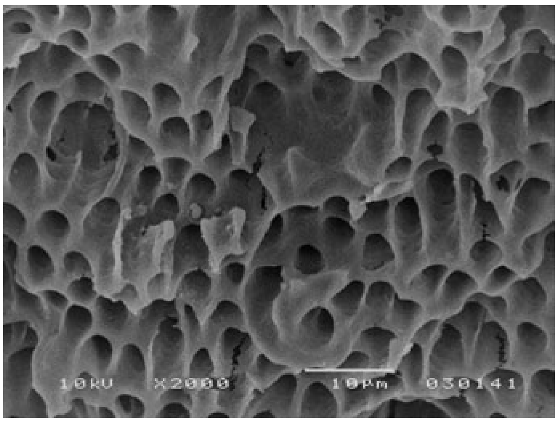

Figure 10 In several samples dentine presented a typical peritubular and intertubular erosion morphology, probably due to EDTA action (group M). Tubular opening had a funnelled appearance. Dentinal erosion sometimes cojoined two or more tubular orifices.

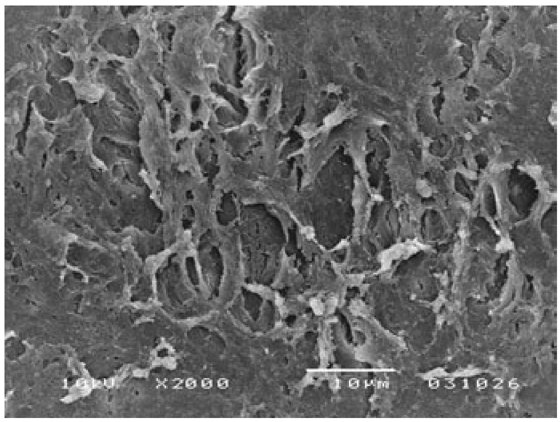

Figure 12 At apical third some samples presented pulpal remnants with collagenous matrix spread along the root canal walls (group P).

Discussion

The present study compared the cleaning efficacy of M*two* and ProTaper NiTi rotary instruments. M*two* has been recently introduced and is characterized by constant taper increasing from size 10 with a 0.4 taper to a size 20 with a 0.6 taper. On the contrary, ProTaper instruments have multiple and progressively changing tapers along the length of their cutting blades; it has recently been investigated for its mechanical properties (Berutti *et al.* 2003, Blum *et al.* 2003). In this study, both instruments were evaluated in partial accordance with the manufacturer's directions. Irrigation procedures were standardized for both experimental groups.

Despite differences being observed, this study demonstrates that both NiTi instruments produced a similar dentine surface on root canal walls for all parameters considered.

SEM analysis demonstrated a substantial portion of dentine surface was free from smear layer; indeed, smear layer was observed only in limited areas of the apical third (Ahlquist *et al.* 2001). Despite some minor differences, both instruments were able to remove smear layer produced during instrumentation and subsequently dissolved by EDTA.

The study confirmed that the apical third has a smaller number of dentinal tubules with a reduced diameter that were only partially covered by a thin smear layer. Peritubular and intertubular erosions

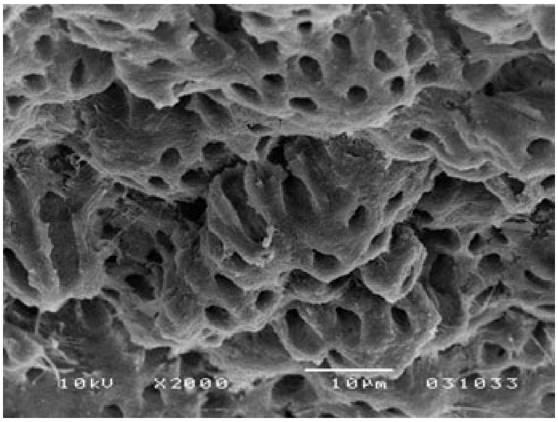

Figure 13 Uninstrumented areas characterized by the presence of predentine (calcospherites) were observed in correspondence of grooves and depressions of the root canal walls (group M).

were frequently observed in many tubule orifices in the middle third mainly in the M*two* group. An irrigation regimen similar to the methodology of Niu *et al.* (2002) was used. The combination of NaOCl and EDTA was probably responsible for the removal of smear layer and for the removal of a great portion of circumferential dentine collagen and mineralized dentine wall from the most superficial part of tubules, as confirmed by a recent review on clinical implications of smear layer (Mayer *et al.* 2002, Torabinejad *et al.* 2002). Previous SEM studies investigated the effect of NiTi rotary instruments on dentine without EDTA (Ahlquist *et al.* 2001, Schafer & Lohmann 2002, Jeon *et al.* 2003, Schafer & Schlingemann 2003) or with the use of EDTA gel as lubricant (Hülsmann *et al.* 2001, Versümer *et al.* 2002). All these studies reported the presence of a greater amount of smear layer after the use of NiTi rotary and stainless manual instruments.

Pulpal debris consisted of small portions of pulpal tissue that were spread on dentine walls during mechanical instruments. This debris was observed only in the apical third where irregularities in canal walls, such as grooves, depressions and large pits, prevented instrument contact. In few samples, pulpal debris was spread along the canal wall, creating a compacted connective network which covered the dentine surface. SEM inspection showed inorganic debris in the apical third. This debris consisted of pieces of dentine (Schafer & Schlingemann 2003) and predentine removed from the inner dentine walls by the mechanical instrumentation. Considering the dimension of dentine fragment/debris (greater than 15–20 microns), irrigant solutions may only partially contribute to their removal from the root canal space. The present study confirms that the apical third is the area where more debris is still visible under SEM inspection (Prati *et al.* 1994). Recent investigations reported partially uninstrumented area with remaining debris in all canals (Schäfers & Zapke 2000, Versümer *et al.* 2002, Schafer & Schlingemann 2003).

Predentine, dentine grooves and depressions were observed in the apical thirds in both groups. Their presence suggests that several areas of dentine were not cut and shaped by these instruments. It is possible that the greater number of wall irregularities of this portion of canal such as depression and grooves may be responsible for the presence of uninstrumented areas. Wu *et al.* (2002, 2003) found that in the apical third the first binding file failed to touch the walls in 25% of curved canals. The use of a larger master files would be more liable to remove a greater portion of debris from the apical thirds with the removal of inner dentine.

It is likely that both NiTi rotary instruments produced fine dentine particles and shavings that were spread and compacted along the dentine wall and then partially dissolved by EDTA and removed coronally via flute spaces. Several studies have suggested a similar mechanism (Jeon *et al.* 2003) for different rotary instruments.

Conclusion

The use of M*two* and ProTaper instruments produced a clean and debris-free dentine surface in the coronal and middle thirds; on the contrary, these instruments were unable to produce a dentine surface free from smear layer and debris in the apical third. The presence of deep grooves and depression on dentine walls in the apical third may well explain the presence of less-instrumented areas.

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