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ORIGINAL ARTICLE

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Appearance of the root canal walls after preparation with NiTi rotary instruments: a comparative SEM investigation

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Abstract The aim of this study was to evaluate, in vitro, with scanning electron microscope (SEM), the appearance of root canal walls shaped by three different rotary NiTi techniques and one conventional manual technique in human extracted teeth. Four different instruments were used: K^3, Hero 642, RaCe and K-file. Each sample was irrigated with 5 ml of 5% NaOCl and 5 ml of 3% H_2O_2 and EDTA, Rc-Prep (1 ml). Each sample was prepared for SEM observation and analyzed in the coronal, middle and apical third, comparing its aspect with a predefined scale of four different parameters: presence of *smear layer, pulpal debris, inorganic debris* and *surface profile*. The apical third showed significantly more pulpal and inorganic debris, smear layer and a high number of surface profile irregularities. No significant difference was found at the coronal, middle and apical thirds between manual and rotary techniques for inorganic debris, smear layer and surface profile. Much pulpal debris were found in the apical third for K^3 and RaCe compared with Hero 642 and K-file. In conclusion, mechanical rotary techniques with NiTi instruments produced quite similar results compared with a conventional manual technique using K-files. The present study demonstrated that dentin and pulpal debris, the morphology of smear layer and surface profile were only partially influenced by the type of endodontic instruments. The apical third was the anatomical area with greater amount of debris and smear layer.

Keywords Dentin · NiTi rotary instruments · Root canal preparation · SEM · Smear layer

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Introduction

Recent studies have clearly documented the presence of bacteria inside the endodontic space and in the dentinal tubules [19]. Many of these bacteria may be incorporated completely or only partially in pulpal and dentinal debris, in the smear layer and inside smear plugs [11]. As demonstrated by many investigations, smear layer is created by endodontic instruments and may be altered by irrigant solutions used during the endodontic procedures [3, 6, 7, 10, 18]. The composition of the endodontic smear layer is still debated, but it may be considered as constituted of dentinal collagen, pulpal debris, bacteria and inorganic debris such as apatite [17, 25]. Different endodontic systems and procedures may produce a different amount of debris and a different morphology of smear layer [9, 18, 20].

The new nickel-titanium (NiTi) instruments may represent a new approach to obtain a rapid endodontic preparation [4, 27]. The type of smear layer produced by NiTi instruments, the removal of dentin and pulpal debris and the quality of endodontic preparation still need to be assessed. In fact, no complete information is yet available on the effect of several NiTi instruments on canal dentin walls.

The aim of this study was to evaluate the ultrastructural morphology of apical, middle and coronal thirds of root canal walls in extracted teeth with straight roots, using different rotary NiTi instruments in association with the crown-down rotary pressureless technique. Conventional manual stainless steel instruments used in association with the crown-down pressureless technique were used as comparative technique [13]. The quality and the amount of smear layer, the amount of pulpal and dentin debris and the ultrastructural surface profile of the canal walls were considered as parameters for the evaluation of the efficacy of instruments.

Materials and methods

Selection of samples

Forty-eight maxillary incisors with straight or slightly curved roots were selected from a pool of recently extracted teeth that were stored in saline solution at 4°C until experimental procedures. All teeth were decoronated with diamond bur under water spray cooling. The working length was determined by measuring the length of #15 K-file passively inserted into the canal until visible at the apical foramen. Each root was evaluated with a #15 K-file for the direct assessment of patency, working length and root canal shape. Teeth with severe elliptical-shaped canals were excluded. In each sample two longitudinal grooves on the palatal/lingual and buccal surfaces of the root were prepared with a diamond bur to facilitate vertical splitting by chisel after instrumentation and to prevent further contamination.

Endodontic preparation

Samples were divided randomly into four groups which were prepared with different instruments: 1) ten teeth with crown-down pressureless mechanical rotary technique and NiTi K^3 instruments (Kerr, San Diego, CA, USA); 2) ten samples with crown-down pressureless mechanical rotary technique and NiTi Hero 642 instruments (Micromega, Besançon, France); 3) ten samples with crown-down pressureless mechanical rotary technique and NiTi RaCe instruments (FKG, La Chaux-de-Fonds, Switzerland); and 4) eighteen samples with crown-down manual technique and K type instruments (FKG, Chaux-de-Fonds, Switzerland). Manual technique with K type instruments was considered as the control group. In each group 5 ml of 5% NaOCl (Niclor 5, Ogna, Maggiò, Italy) and 5 ml of 3% H_2O_2 solutions (Ogna, Maggiò, Italy) and approximately 1 ml of RC Prep EDTA gel (Premier, Philadelphia, PH, USA) stored at room temperature were used as irrigation and lubricant systems. Irrigation was performed with an endodontic needle, with a diameter of 30 gauges (0.25 mm) (Molteni Jet APS, Molteni dental, Scandicci, Italy), first with the 3% H_2O_2 solution, and then followed by a second irrigation with the 5% NaOCl solution. The irrigation regime was performed after each change of instrument, dividing equally the total amount of irrigant according to the number of instruments present in the different techniques. During the instrumentation procedures, great care was taken to include, as additional lubricant gel, RC-Prep as far as the apex of each sample, using a manual K-file #10 in an anticlockwise manner.

A low-speed (300–400 rpm) rotary endodontic handpiece was used for NiTi instruments (WA-62 LT, W&H WEHADENT Salzburg, Austria). Each NiTi instrument was used for no more than five different canal preparations.

K^3

The sequences used in this study were proposed by the manufacturer for slightly curved canals (curvature gauge <5°): .10 taper (Orifice Opener) for the coronal third; .08 taper (Orifice Opener) for the coronal third; .06 taper (K^3) size 40, at approximately 2/3 of working length (WL); .06 taper (K^3) size 35, at approximately 2/3 of WL+1 mm; .06 taper (K^3) size 30, at WL–3 mm; .06 taper (K^3) size 25, at WL.

Hero 642

The sequences used in this study correspond to the manufacturer's instruction for slightly curved canals (curvature gauge <5°): 6% taper, size 25 at approximately 2/3 of WL; 4% taper, size 25 at WL–2 mm; 2% taper, size 25 at WL.

RaCe

The sequences used in this study were proposed by the manufacturer for slightly curved canals (curvature gauge <5°): .10 taper (PRE-RaCe) size 40 at approximately 2/3 of WL; .08 taper (PRE-RaCe) size 35 at approximately 2/3 of WL+1 mm; .06 taper (PRE-RaCe) size 30 at WL–3 mm; .04 taper (RaCe) size 25 at WL–2 mm; .02 taper (RaCe) size 25 at WL.

K-file

Previously the coronal third was opened with Gates Glidden Burs (#4-#3-#2). K-file instruments were used in the coronal and middle thirds in the following order: #60, #50, #40, #35, #30, #25. The apex was instrumented with K-File #10, #15, #20 and #25.

SEM evaluation

After preparation, each sample was immediately 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 at 4°C, dehydrated in graded concentration alcohol, air dried, then gold sputtered and observed at SEM (JEOL 5200, JEOL, Tokyo, Japan). SEM observations were obtained, for statistical analysis, at a standard magnification of ×2,000. Six microphotographs were taken at each third (coronal, middle and apical) in standard positions, measured with SEMafore PC program (JEOL, Tokyo, Japan) and rated in double blind method by two trained operators. Specific areas of dentin were observed for qualitative analysis at greater magnification (×5,000, ×10,000, ×15,000).

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; tubules completely opened	Present in limited areas, less than 75% of tubules uncovered; tubules partially opened	Present, tubules visible in limited areas and partially closed; less than 50% of dentinal tubules visible	Homogeneous smear layer present above all dentin; dentinal tubules not visible
Pulpal debris	Absent	Minimal presence of pulpal-fibrous debris	Partial presence of pulpal-fibrous debris	Presence of an organised collagenous matrix
Inorganic debris	Absent	Minimal presence	Often present	Present everywhere and covering dentin surface
Surface profile	Absence of irregularities	Isolated irregularities and grooves	Partially irregular, with limited non-instrumented areas	Irregular with grooves, areas of non-instrumented dentin

Scoring system

Three different areas (coronal, middle and apical third) were analyzed comparing the morphology of dentin surface with a predefined scale of values which considers four different parameters, *smear layer, pulpal debris, inorganic debris, surface profile*, in order to allot a score to all the areas of each specimen [9, 18, 21, 23, 24]. For the correct dentin profile, the presence of grooves, pits and predentin areas were evaluated. Table 1 shows the scale of values for the four different parameters evaluated.

Statistical analysis

The Kruskal Wallis test (ANOVA) for non parametric data was used for all data analysis. Box and whiskers plots were drawn. Horizontal lines, called whiskers, extend from each end of the box. The lower (left) whisker is drawn from the lower quartile to the smallest point within 1.5 interquartile ranges from the lower quartile. The other whisker is drawn from the upper quartile to the largest point within 1.5 interquartile ranges from the upper quartile. A median notch is placed around the media to indicate an approximate confidence interval for the median. The length of the notch represents an approximate 95% confidence interval for the median. Notches that overlap indicate that there is no significant difference between the sample median.

Results

Smear layer

Results from the Kruskal-Wallis test showed a significant difference among the three areas observed (Test statistic = 36.7; p <0.01). In all groups the apical third had a greater amount of smear layer as compared with the coronal and middle third. There was also no significant difference between coronal and middle thirds. No significant difference was found among the four groups of instrumentation (Test statistic = 1.7; ns). K^3 showed a greater amount of smear layer at apical third (Fig. 1) compared with coronal and middle third (Test statistic = 13.1; p <0.01). Hero 642 presented a greater amount of smear layer both at apical and middle third compared with coronal third, while no significant difference was found between middle and apical third (Test statistic = 9.5; p <0.01). RaCe showed no significant difference among the three areas (Test statistic = 5.4; ns), while K-file showed a significant difference among coronal, middle and apical third, with an increasing presence of smear layer from coronal to apical third (Test statistic = 10.7; p <0.01) (Fig. 2).

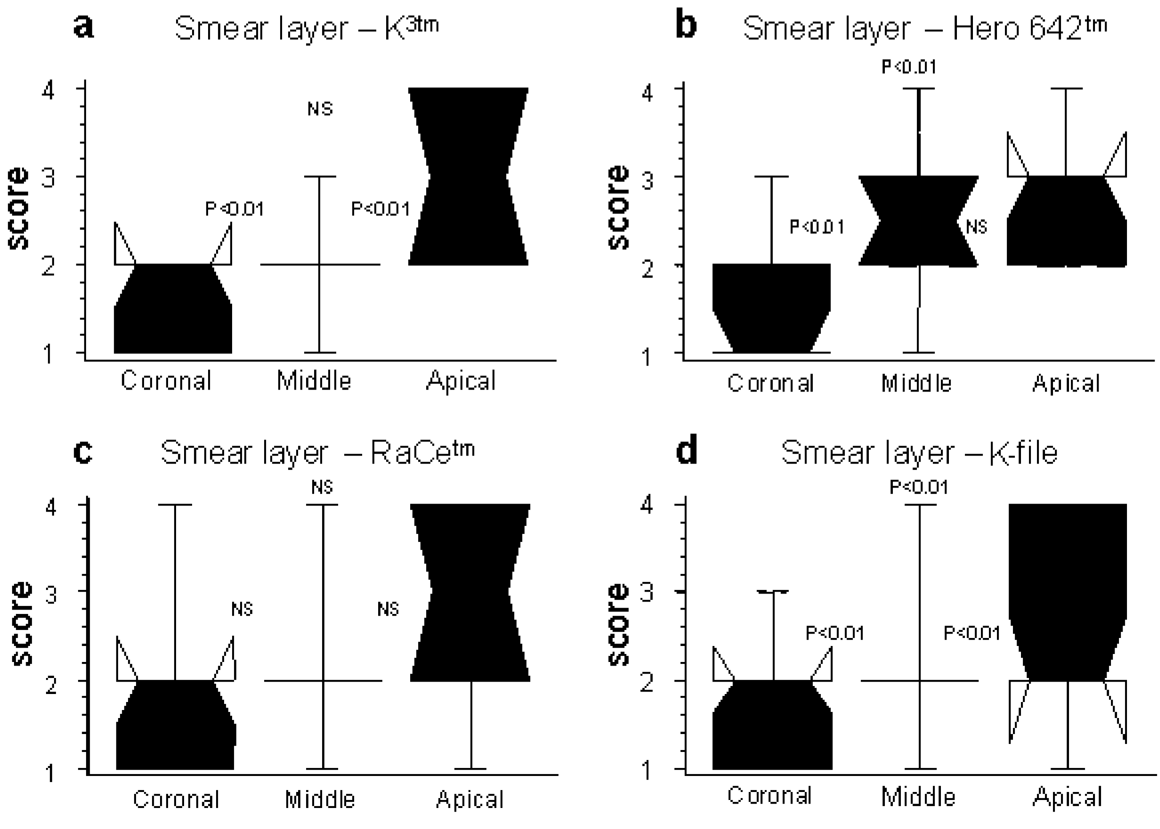

Fig. 1a–d Box-and-whisker plot of smear layer parameter relating to the area of observation for each instrument: (**a**) K^3; (**b**) Hero 642; (**c**) RaCe; (**d**) K-file

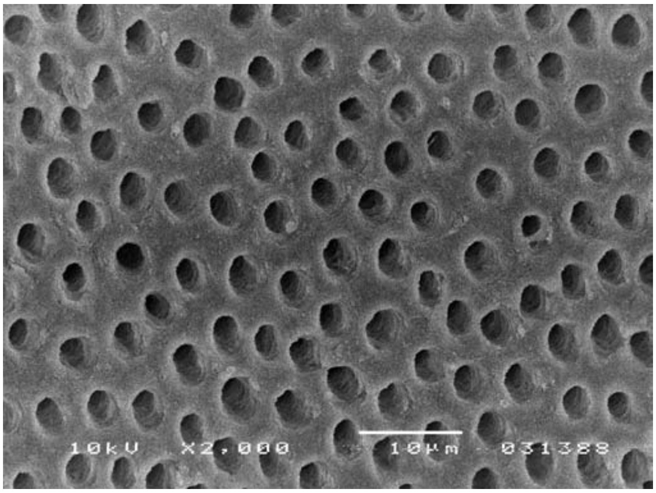

Fig. 2 Coronal third. Dentin surface after preparation with K-file. Smear layer is absent and dentinal tubules are visible. Smear plugs do not close the orifices of dentinal tubules. Smear layer score = 1

Inorganic debris

The apical third presented a greater amount of inorganic debris compared with coronal and middle third (Test statistic = 55.9; p <0.01). No difference was found between coronal and middle third.

No statistical difference was found between the four groups of instruments (Test statistic = 2.7; ns) (Fig. 3). K^3 produced a significantly greater amount of inorganic debris at apical third compared with coronal and middle third (Test statistic = 11.7; p <0.01). As regards Hero 642, no significant difference was found among coronal, middle and apical third (Test statistic = 5.7). RaCe showed a significant difference among coronal, middle and apical third, with an increasing presence of inorganic debris (Test statistic = 13.0; p <0.01). K-file showed significantly less inorganic debris in the coronal third when compared with middle and apical third if these are taken together (Test statistic = 28.9; p <0.01), while no difference was registered between the coronal and middle third and between middle and apical third.

Pulpal debris

Also for this parameter, apical third presented a greater amount of pulpal debris compared with coronal and middle third (Test statistic = 23.8; p <0.01) (Fig. 4). No significant difference was found between coronal and middle thirds.

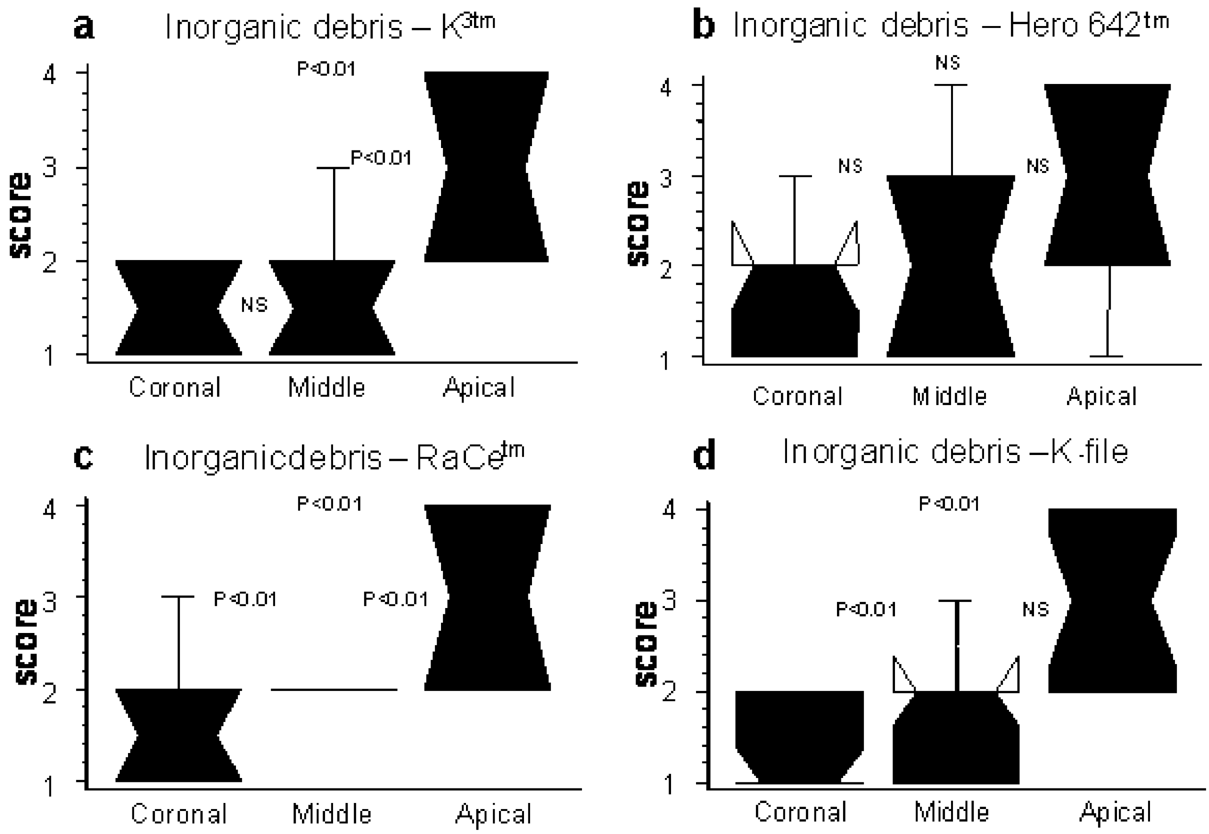

Fig. 3a–d Box-and-whisker plot of inorganic debris parameter relating to the area of observation for each instrument: (**a**) K^3; (**b**) Hero 642; (**c**) RaCe; (**d**) K-file

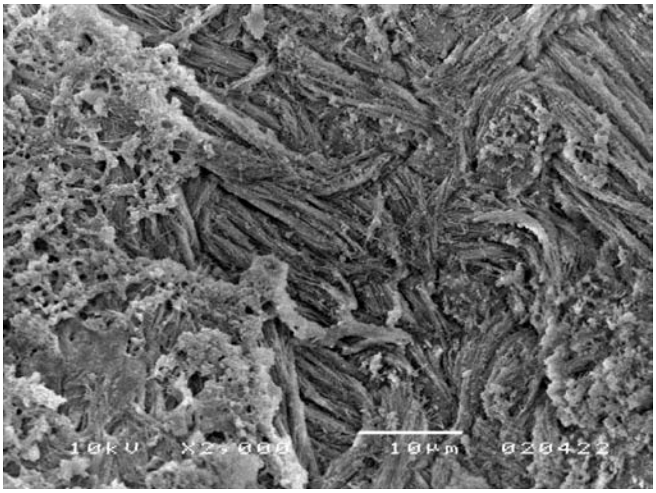

Fig. 4 Pulpal debris were visible only in the apical third of a few samples. These debris were packed and spread along the dentin walls. Many dentin debris were trapped into the collagen-pulpal debris network, as demonstrated by the figure

Contrary to previous parameters, a statistically significant difference between instruments was found here (Test statistic = 18.0; p <0.01). The K-file group presented a lower amount of pulpal debris compared with the other instruments. No difference was found at coronal third among the four groups (Test statistic = 5.8; ns). At middle third, all NiTi instruments (K^3, Hero 642, RaCe) produced a greater amount of pulpal debris (Test statistic = 7.6; p <0.05). At apical third, K^3 produced a significantly greater amount of pulpal debris compared with RaCe and K-file (Test statistic = 13.1; p <0.01). No differences were found among Hero 642, RaCe and K-file. The interaction between each single instrument and the area of observation (Fig. 5) demonstrated that K^3 left a greater amount of pulpal debris at apical third compared with coronal and middle third (Test statistic = 8.7; p<0.01). Similar results were found for Hero 642 (Test statistic = 13.1; p <0.01). RaCe presented a greater extent of pulpal debris at apical third compared with coronal third (Test statistic = 5.9; p <0.05). On the contrary, the K-file instrument showed a similar amount of pulpal debris in the three areas of observation (Test statistic = 5.2; ns).

Surface profile

Also for surface profile parameter, a significant difference was detected as regards the anatomical position of root canal space (Test statistic = 36.7; p <0.01). At the apical third, surface profile was affected by the presence of grooves and uninstrumented area with respect to middle

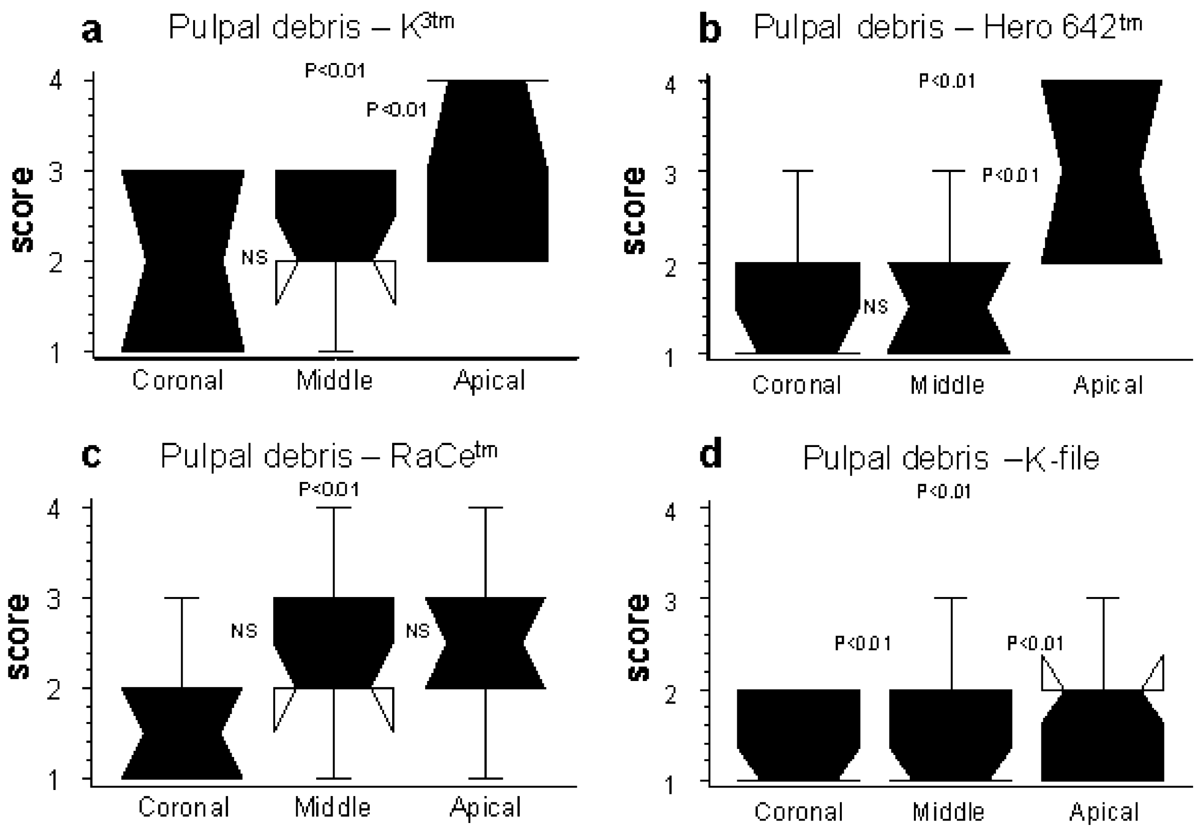

Fig. 5a–d Box-and-whisker plot of pulpal debris parameter relating to the area of observation for each instrument: (**a**) K^3; (**b**) Hero 642; (**c**) RaCe; (**d**) K-file

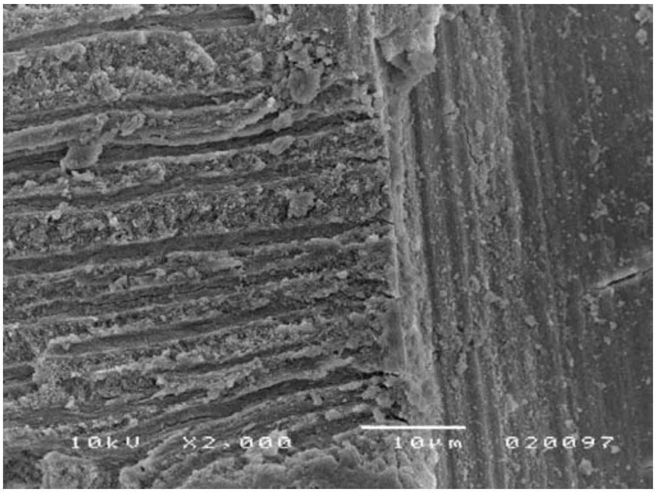

Fig. 6 Cross-section of dentin. At apical third many irregularities were visible on dentin surface

and coronal third (Fig. 6). No difference was registered between coronal and middle third. No significant difference was found either concerning the kind of instrument (Test statistic = 1.7; ns) or the interaction between instrument and position (coronal third test statistic = 1.3; middle third test statistic = 1.3; apical third test statistic = 2.4; ns). The interaction between each single instrument and the area observed (Fig. 7) demonstrated in the K^3 group a greater presence of unprepared areas, predentin and grooves at the middle and apical thirds compared with the coronal third (Test statistic = 13.2; $p < 0.01$). Hero 642 results showed a significant difference between the middle and apical third compared with the coronal third (Test statistic = 9.6; $p < 0.01$). No difference was found between the middle and apical thirds. Concerning RaCe, no significant difference was detected among the different areas observed (Test statistic = 5.4; ns). The K-file group showed a greater presence of unprepared areas, predentin and grooves at the middle and apical thirds compared with the coronal third (Test statistic = 10.8; $p < 0.01$).

Discussion

All techniques tested in this study produced smear layer (Fig. 8) and smear plugs that partially occluded dentinal tubules and created 1–5 µm long debris tags (smear plugs) that completely filled dentinal tubules, as shown by longitudinal SEM pictures. For this reason we have evidence that all the instruments were able to cut dentin surface and produced fine dentin debris. It is well known

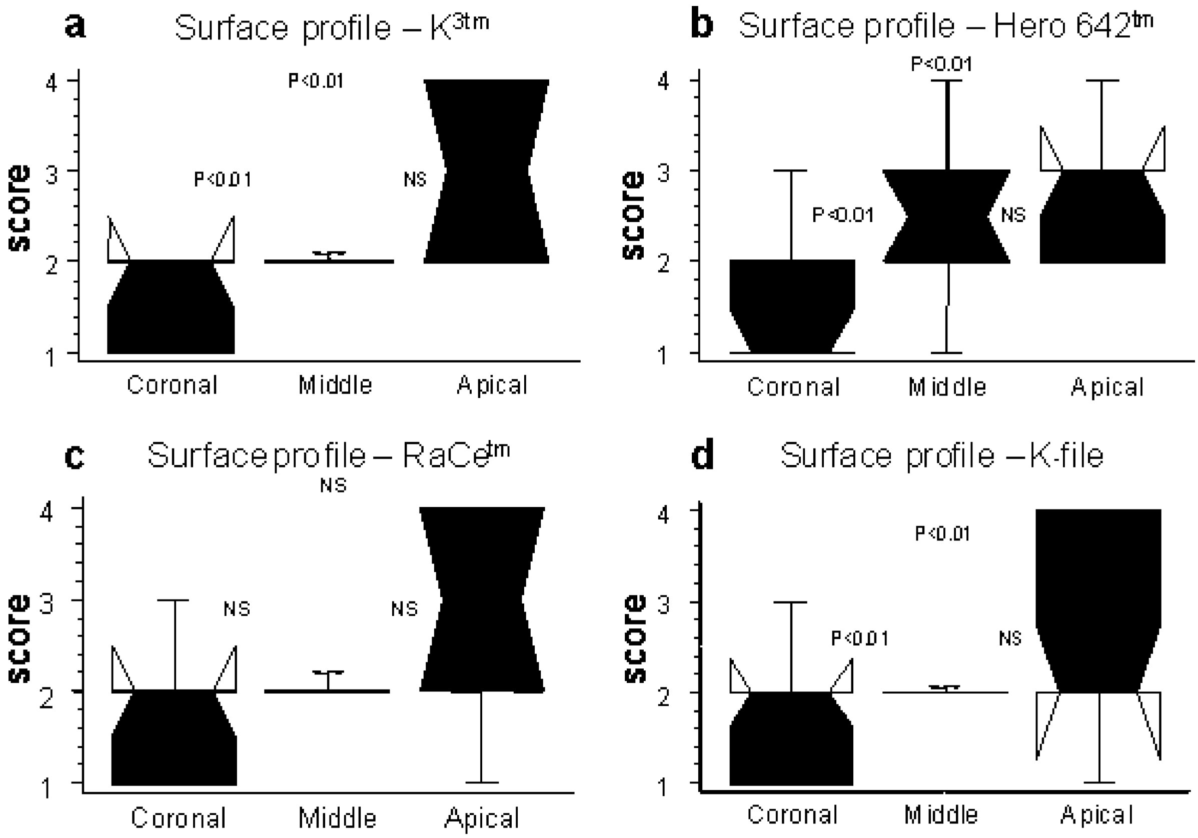

Fig. 7 Box-and-whisker plot of surface profile parameter relating to the area of observation for each instrument: (**a**) K^3; (**b**) Hero 642; (**c**) RaCe; (**d**) K-file

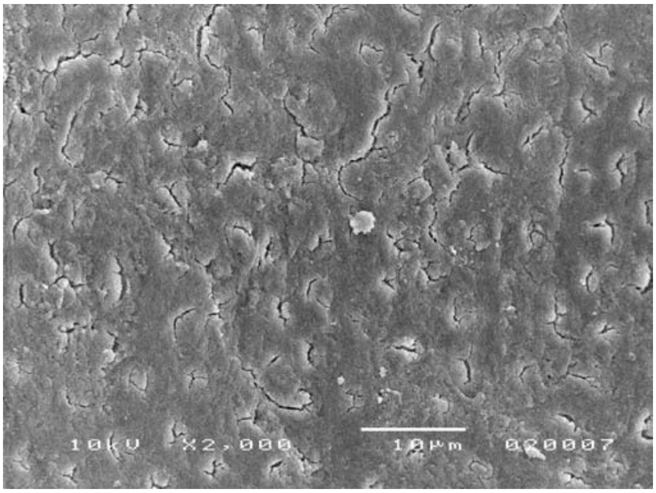

Fig. 8 Coronal third. Dentin treated with RaCe NiTi instruments. Smear layer partially masks tubules orifices. Smear layer score = 3

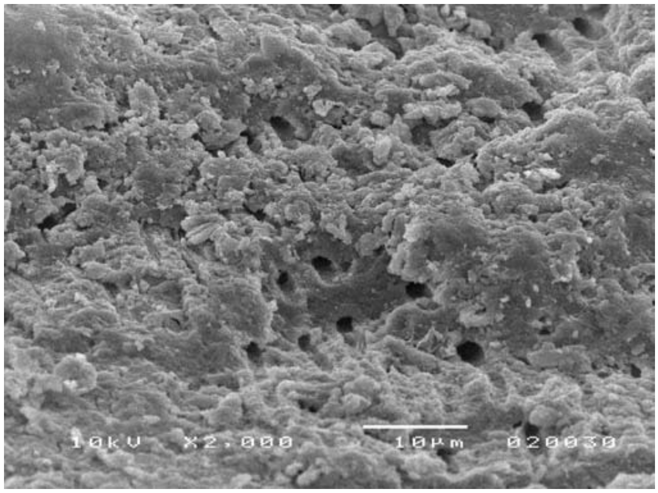

Fig. 9 Smear layer spread on dentin surface, with a configuration called “tree bark”. Several open dentinal tubules are visible just below the layer of dentin debris

that smear layer may be created by several instruments such as diamond and steel burs, curettes, sandpaper, diamond paste, etc. [7, 17, 21]. From this study it is evident that these endodontic instruments create a great amount of fine debris during their mechanical abrasion of dentin walls. The mean dimension of fine smear layer particles has been previously reported to be approximately 0.05–0.1 µm [15, 16, 17]. Hence, NaOCl irrigant may modify the composition of smear layer and morphology of dentin surface by removing dentin collagen [22] and is able to dissolve and remove large portions of pulpal tissue [2, 12, 18]. After this irrigation, the endodontic smear layer is, therefore, different from the one created during a conventional cavity preparation because it is completely free from collagen and other proteinaceous components that are partially removed [25]. However, our study demonstrated that several areas with pulpal debris were still present after use of different instruments. This problem was observed in all the groups of instruments, suggesting that all the techniques were unable to completely remove pulpal debris from dentin grooves and depression. We presumed that the morphology of the endodontic smear layer may be greatly affected by design of instrument and methods of application [8, 12, 20, 23] and, obviously, by the type of irrigation [3, 12, 14, 18]. Our study demonstrated that the three mechanical instruments produced quite similar smear layer morphology. On the contrary, K-file manual instrumentation produced a fine multilayered smear layer, the so-called tree bark smear layer, as previously described [21] (Fig. 9). The study supports the hypothesis that rotary NiTi instruments create different ultrastructural aspects on dentin walls as compared with manual stainless-steel K-files. At the apical third a great number of fine porosities was observed in the top of smear layer. Probably the use of EDTA gel (RC-Prep) and irrigant solutions increased the ability of NiTi instruments to remove the new smear layer and to reduce its thickness, facilitating its removal.

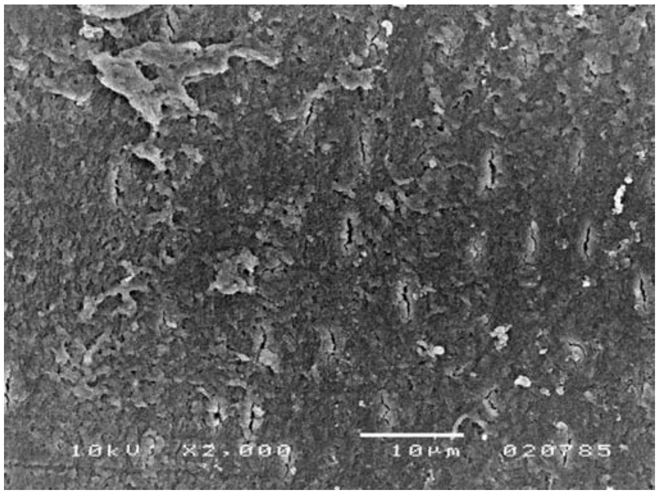

Fig. 10 Apical third. Dentin treated with Hero 642 NiTi instruments. A homogeneous smear layer is present and covers dentinal tubules. Also, pulpal debris are visible. Smear layer score = 3

However, it is probable that only a small amount of RC-Prep may reach the apical third due to the high viscosity of the gel. For this reason, the removal of the new smear layer from the apical dentin was limited in many samples (Fig. 10). It is also possible that the greater amount of dentin (and pulpal) debris at the apical third may contribute to the formation of a thick and compacted smear layer extremely difficult to be solved and removed. The problem of presence/absence of smear layer is extremely important considering its possible role in preventing apical fluid flow, apical sealing and bacterial contamination of dentinal tubules [6, 11, 26, 28]. It may also be supposed that smear layer plays a negative role, inducing bacteria contamination and preventing better adaptation of cements and endodontic sealers [26]. On the other hand, smear plugs may be responsible for reduced permeability and play a protective role in reducing permeability of root canal walls and in preventing bacterial infiltration [5, 11]. These speculations need still to be confirmed in the future. We suppose that the long time used to instrument each canal (30–40 min) kept NaOCl in contact with dentin walls for sufficient time to remove the exposed collagen fibrils, as observed in almost all of our SEM pictures. These data suggest that during instrumentation with NiTi the production of smear layer (and its removal) allows a correct fluid-irrigation movement inside canal.

Pulpal debris consisted of small portions of pulpal tissue that were probably compacted and spread out on the canal walls by mechanical instruments. In our study, pulpal debris were extremely rare and observed only at apical third when irregularities of canal walls such as grooves, depressions and large pits prevented adequate shaping and cleaning. So apparently, the NaOCl did not get with sufficient volume in areas not readily accessible. It is also possible that the packing of pulpal tissue along the canal walls may create a collapsed layer of collagen, fibroblasts and other non-collagenous proteins that were difficult to be removed by NaOCl. Differences were observed between the groups tested, suggesting that not only irrigant solutions are important in determining the morphology of root canal walls, but also the type of instrument used. Also, a recent investigation confirms that several NiTi instruments are partially unable to remove debris from the root canal [1].

All systems produced a considerable amount of dentin debris. SEM inspection showed debris at the apical and middle thirds. The dimensions (5–40 μm) suggest that these debris consist of pieces of root canal walls and probably fragments of predentin and are produced by mechanical instrumentation. Probably most of them may be removed by flushing a larger volume of irrigant solutions [12, 21]. Our study confirms that the apical third is the area where more debris are still visible under SEM inspection. As regards surface profile, the presence of predentin and canal grooves has been previously described [18]. Their presence suggests that the endodontic anatomical irregularities (voids, depressions, predentin areas) may greatly influence the regularity and morphology of canal walls and may affect the canal sealing. To remove these irregularities, in clinical practice, it is absolutely necessary to increase the cutting and the shaping ability of instruments and the diameter of the endodontic canal. However, this procedure may increase the risk of a greater and dangerous removal of dentin wall, leading to immediate perforation or late fracture of the root. The use of a crown-down method probably limited the presence of non-instrumented predentin only to apical thirds.

All the instruments were unable to completely create a homogeneous surface profile in the apical third with respect to the other areas (medium and coronal) as demonstrated by other studies [1, 23]. SEM observations demonstrated that the coronal third may be completely instrumented and kept free from pulpal and inorganic debris. No areas of unprepared dentin were detected. On the contrary, the apical and middle thirds showed more pre-dentin. Hence, several unprepared dentin areas were detected at these thirds. Peters and Barbakow [18] reported several unprepared areas at the coronal third. In our study the unprepared area and predentin were located only at the middle and apical thirds. However, the different techniques used by the authors may well explain these differences.

The study suggests that morphology of smear layer was not in relationship with the NiTi flute design and technique of use. Anatomical conditions and position may reduce effectiveness of RC-Prep and NaOCl irrigants that probably mask the effect of the instruments on dentin surface.

In conclusion the morphology of debris, of smear layer and the amount of collagen fibrils still present on the canal walls may give more information on the quality of dentin preparation. The use of new NiTi instruments may contribute to a preparation of canal walls only partially different from K-file with manual technique. The apical third was the critical area where a greater number of debris is located. Finally, the use of an index to determine the surface profile may contribute to a better evaluation of the effectiveness of endodontic instruments on root canal dentin.

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