

SEM EVALUATION OF ROOT CANAL WALLS AFTER HYBRID PREPARATION WITH NiTi ROTARY INSTRUMENTS AND FOUR DIFFERENT IRRIGATION TECHNIQUES

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Abstract

Objectives To evaluate the appearance of root canal walls *in vitro*, after hybrid preparation with NiTi rotary instruments and one of four irrigation regimes, using scanning electron microscopy (SEM). The null hypothesis stated that no differences in debris on canal walls and surface morphology should be observable between the experimental groups after the different irrigation techniques.

Materials and methods Forty single-rooted human teeth (incisors and canines), freshly extracted for periodontal reasons, were divided into four groups, endodontically prepared, and irrigated as follows:

- Group 1: 5% sodium hypochlorite during preparation plus final flush with 5% sodium hypochlorite at 40°C;
- Group 2: 5% sodium hypochlorite during preparation plus final flush with 5% sodium hypochlorite and 15% liquid EDTA at 40°C;
- Group 3: 5% sodium hypochlorite and viscous EDTA during preparation plus final flush with 5% sodium hypochlorite at 40°C;
- Group 4: 5% sodium hypochlorite and viscous EDTA during preparation plus final flush with 5% sodium hypochlorite and 15% liquid EDTA at 40°C.

Canal walls were assessed and compared using a predefined scale of four parameters: smear layer, pulpal debris, inorganic debris and surface profile. Data were evaluated statistically using the Kruskal-Wallis one way ANOVA test.

Results A statistically significant difference ($p < 0.05$) was found for groups 2 and 4 when compared with groups 1 and 3 for presence of smear layer, pulpal debris, inorganic debris and surface profile. In group 3 samples, the apical third presented a surface profile affected by unprepared regions in which predentin was still visible.

Conclusions The combination of sodium hypochlorite and liquid EDTA was effective in cleaning the root canal system, but was unable to produce a dentin surface free from debris in the apical third.

Running title: Cleaning ability of four irrigation techniques.

Keywords: EDTA, NiTi rotary instruments, root canal preparation, SEM, smear layer, sodium hypochlorite.

Introduction

One of the most important procedures during root canal treatment is the chemo-mechanical preparation of the root canal, based on the correct use of instruments and irrigating solutions (Walton & Torabinejad 1996, Gambarini 1999).

The latest nickel-titanium (NiTi), variable taper, rotary instruments are a relatively new approach to obtain good root canal preparation with standardized taper, in less time than necessary for manual preparation (Gambarini 1999, Foschi *et al.* 2004, Short *et al.* 1997).

As demonstrated by many investigations, dentin canal wall preparation, using either manual or mechanized techniques, always causes the formation of pulpal debris, smear layer and smear plugs, which cover the whole root canal surface (Lim *et*

al. 2003, Torabinejad *et al.* 2002, Jeon *et al.* 2003, Hülsmann *et al.* 2001). The smear layer and plugs consist of an organic portion (pulp tissue debris, odontoblastic process, proteins, saliva, microorganisms and blood cells) and an inorganic portion (minerals from the dentin structure) (Sen *et al.* 1995).

There is no scientific consensus on the efficacy of smear layer removal in root canal treatment. In fact, some scientific studies suggest that the smear layer produced during root canal therapy may slow bacterial movement, although it does not prevent bacterial entrance after treatment (Williams & Goldmann 1985). Drake *et al.* (1994) asserted that the smear layer may inhibit bacterial colonization of the root canal. However, it has also been reported that its removal reduces the bacterial population in the root canal and enhances sealer and gutta-percha obturation properties (Bowman & Baumgartner 2002). For these reasons, scientific consensus is currently in favour of smear layer removal to create the cleanest dentin surface possible, considered an essential step in successful root canal treatment (Peters & Barbakow 2000).

Sodium hypochlorite (NaOCl) is still considered the best available canal irrigant due to its antibacterial and organic tissue-dissolving properties (Naenni *et al.* 2004, Berutti & Marini 1996). However, NaOCl is unable to remove the endodontic smear layer formed on the prepared canal walls (Cunningham & Balekjian 1980, Abou-Rass & Oglesby 1981).

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Table 1. Scale of values assigned to the four morphological parameters evaluated.

	1	2	3	4
<i>Smear layer</i>	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.
<i>Pulpal debris</i>	Absent	Minimal presence of pulpal-fibrous debris	Partial presence of pulpal-fibrous debris	Presence of an organised collagenous matrix
<i>Inorganic debris</i>	Absent	Minimal presence	Often present	Present everywhere and covering dentin surface
<i>Surface profile</i>	Absence of irregularities	Isolated irregularities and grooves	Partially irregular, with limited non-instrumented areas	Irregular with grooves, areas of non-instrumented dentin

Otsby (1957) was the first to propose the use of ethylenediamine tetraacetic acid (EDTA) as an irrigant solution to remove the inorganic component. The association of NaOCl and EDTA solutions has been advocated for the effective removal of soft tissue as well as the inorganic/organic smear layer (Baumgartner & Mader 1987).

The aim of this scanning electron microscopic (SEM) study was to evaluate the presence of smear layer, pulpal debris and inorganic debris on root canal walls after preparation and irrigation with different combinations of liquid EDTA, viscous EDTA and NaOCl.

The null hypothesis stated that no differences in debris on canal walls and surface morphology should be observable between the experimental groups after the different irrigation techniques.

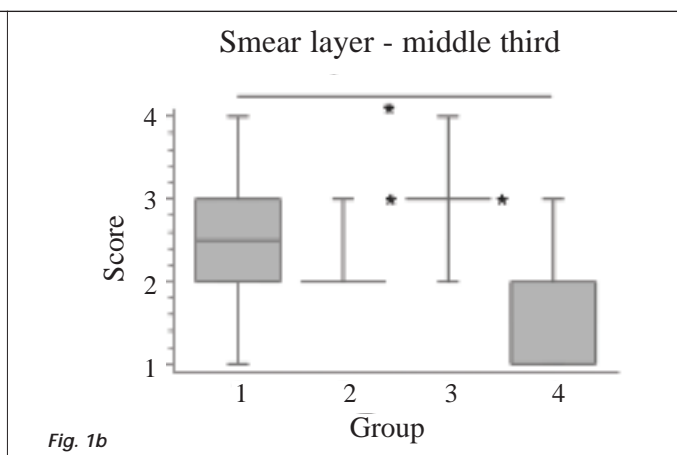
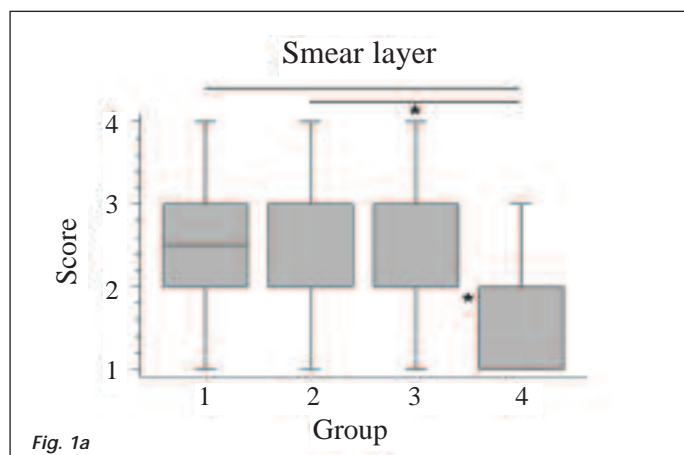
Materials and methods

Sample selection

Forty maxillary anterior teeth, (incisors and canines) each with a single root canal, extracted for periodontal reasons,

checked by radiographs (70 kV and 0.08 seconds), taken in both buccolingual and mesiodistal directions, using a Rinn device (Rinn Corporation, Elgin, Ill), were selected from a pool of recently extracted teeth and stored in saline solution at 4°C until the experimental procedure. The teeth were intact, unrestored and free of caries or visible cracks.

The crown of each tooth was removed at the cementum enamel junction (CEJ) with a diamond bur, changed after twenty crowns' removal, under water spray cooling to ensure good canal visibility and optimal access. To facilitate tooth fracture and prevent further contamination for SEM examination, before preparation two parallel longitudinal grooves, not penetrating the root canals, were made on both palatal/lingual and buccal tooth surfaces with a diamond bur used with a high-speed, water-cooled hand piece. Final working lengths were set by subtracting 1 mm from the lengths recorded when tips of #10 or #15 K-files (Dentsply Maillefer, Ballaigues, Switzerland) were visible at the apical foramina. All working lengths were confirmed radiographically using a Rinn device from buccal view.



Figs. 1a-b Box and whisker plots showing: a) statistical differences between group 4 compared to the all the other three groups for the presence of smear layer; b) significantly higher amount of smear layer at the middle third for group 3 compared to group 4 and 2. *, P < 0.05.

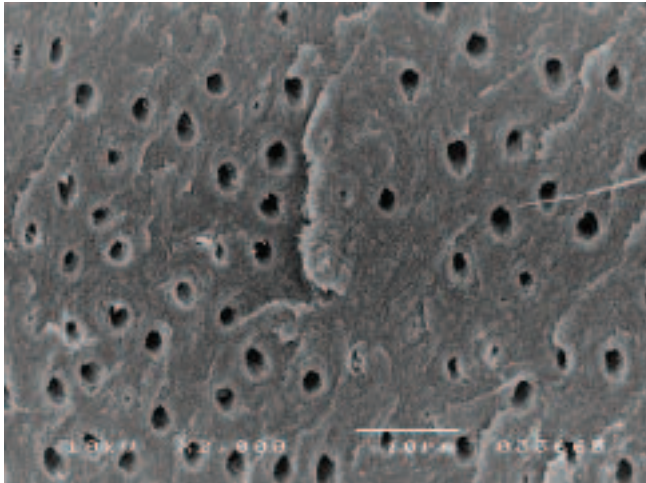


Fig. 2 Micrograph demonstrating the presence of partially opened tubules at middle third. Group 3

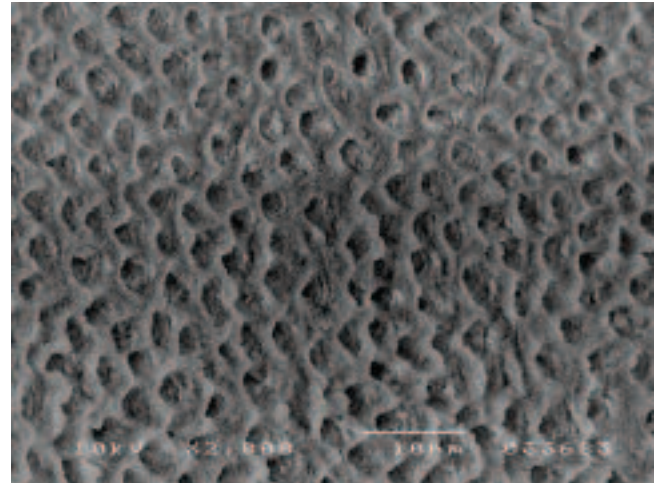


Fig. 3 Micrograph demonstrating the absence of smear layer and visible tubular orifices at middle third. Group 2.

Endodontic preparation

The teeth were randomly divided into four groups (1, 2, 3, 4) of 10 teeth each.

The canals were prepared using Ni-Ti rotary instrumentation. The coronal two thirds were enlarged using ProTaper (Dentsply Maillefer, Ballaigues, Switzerland) with a “crown-down” canal preparation technique. Preliminary coronal enlargement was achieved using ProTaper S1, ProTaper SX, ProTaper S1, ProTaper S2 and ProTaper F1 sequentially. Apical preparations were then completed using Profile .06 #25 (Dentsply Maillefer, Ballaigues, Switzerland) and GT-rotary .06 #30 (Dentsply Maillefer, Ballaigues, Switzerland). After ten root canal preparations, Ni-Ti rotary instruments were changed. Instruments were employed in a controlled, slow-speed, high-torque motor at a continuous speed of 300 rpm. All teeth were prepared by two inter-consistent experts. Root canals were irrigated after use of each instrument and were kept flooded with irrigant during the preparation phase. A syringe with a 27-gauge needle was used for each irrigant. Solutions and methods used to irrigate the root canals were group-dependent:

Group 1 (10 Specimens)

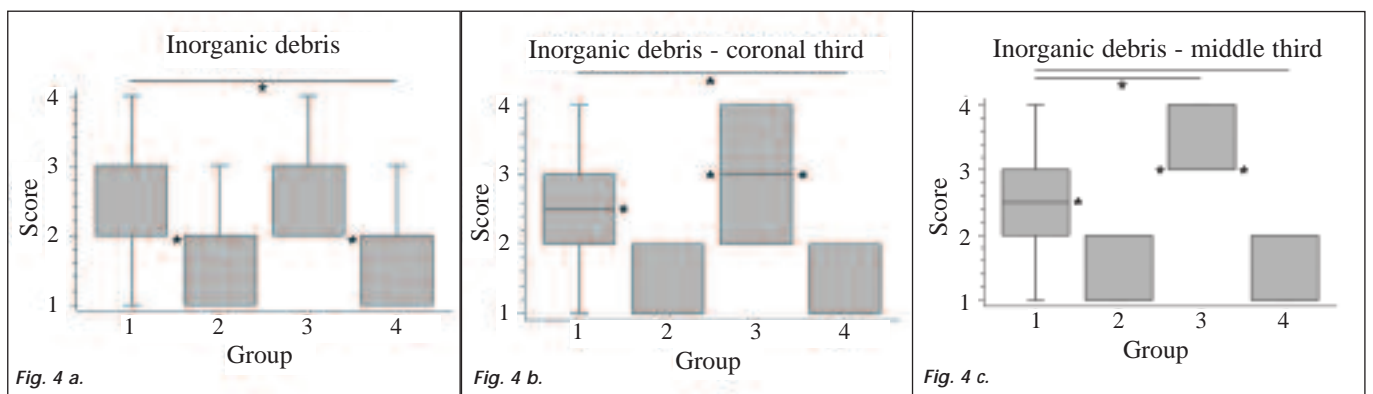
During preparation, teeth were irrigated at room temperature with 1 ml of 5% NaOCl (Nicolor 5; Ogna, Muggiò, Italy) after the use of each rotary instrument; after preparation, the canals were flushed with 1 ml of 5% NaOCl (Ogna) at 40°C for 2 min.

Group 2 (10 Specimens)

During preparation, teeth were irrigated at room temperature with 1 ml of 5% NaOCl (Ogna) after the use of each rotary instrument; after preparation, each canal received a final irrigation with 1 ml of 5% NaOCl (Ogna) at 40°C for 2 min, followed by 1 ml of 15% liquid EDTA (Largal Ultra, Ogna, Muggiò, Italy) for 2 min, immediately followed by 1 ml of 5% NaOCl (Ogna) at 40°C for 1 min.

Group 3 (10 Specimens)

During preparation, teeth were irrigated at room temperature with 1 ml of 5% NaOCl (Ogna) after use of each rotary instrument and 1 ml of viscous EDTA applied with a



Figs. 4 a-b-c. Box and whisker plots showing: a) the overall differences among the four groups for the inorganic debris parameter; b) At the coronal third a significantly higher amount of smear layer was observed in group 3 compared to group 2 and 4. Group 1 presented more inorganic debris compared to group 2 and group 4; c) At middle third group 3 was more affected by the presence of inorganic debris compared to all the other experimental groups. *, P < 0.05.

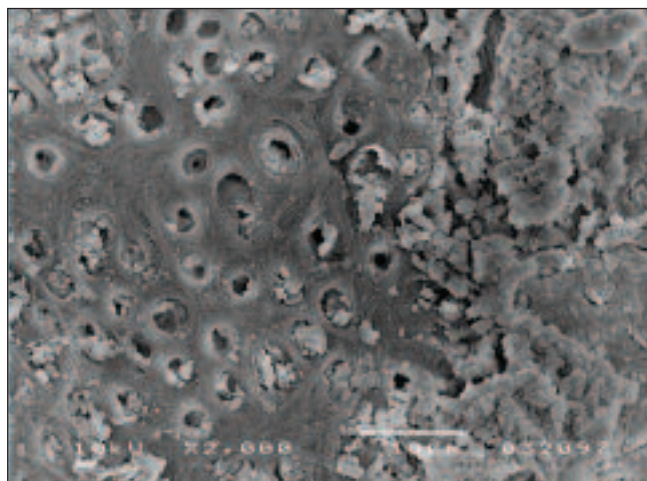


Fig. 5. Micrograph shows the presence of inorganic debris at coronal third. Group 3.

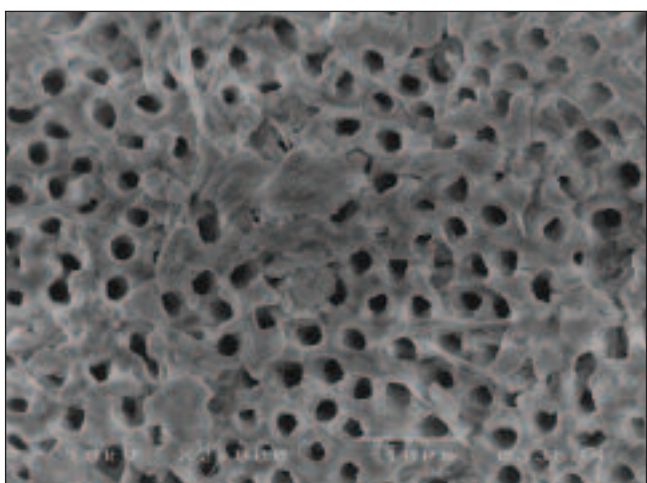


Fig. 6. Micrograph shows the minimal presence of inorganic debris at coronal third. Group 2.

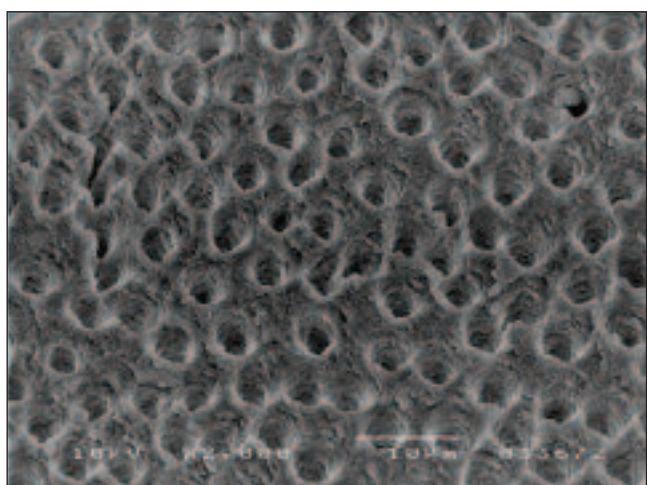


Fig. 7. Micrograph shows the absence of inorganic debris at coronal third. Group 4.

paper point (Glyde File Prep, Dentsply De Trey GmbH, Germany) alternately; after preparation, each canal received a final irrigation with 1 ml of 5% NaOCl (Ogna) at 40°C for 2 min.

Group 4 (10 Specimens)

During preparation, teeth were irrigated at room temperature with 1 ml of 5% NaOCl (Ogna) after use of each rotary instrument and 1 ml of viscous EDTA applied with a paper point (Glyde File Prep, Dentsply); after preparation, each canal received a final irrigation with 1 ml of 5% NaOCl (Ogna) at 40°C for 2 min followed by 1 ml of 15% liquid EDTA (Largal Ultra, Ogna) for 2 min, immediately followed by 1 ml of 5% NaOCl (Ogna) at 40°C for 1 min.

SEM preparation

Immediately after preparation each sample was split in half 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).

Scoring system.

After a general survey of the canal wall from the apex to the crown, six SEM microphotographs were obtained at a standard magnification of 2000x at each third (coronal, middle and apical). A total of eighteen microphotographs per specimen were recorded. Specific areas of dentin were observed at greater magnification (5000x).

The images were saved digitally with specific software (SemaFore-JEOL, Tokyo, Japan) and rated in a double blind method by two trained operators.

Four parameters were analyzed to assess the microscopic root canal space morphology: *smear layer*, *pulpal debris*, *inorganic debris* and *surface profile*. Each image was rated double-blind by two trained operators, according to a four value scale (Table 1) presented in a previous study (Prati *et al.* 2004). Where there was a discrepancy between the two ratings, the lower score was assigned.

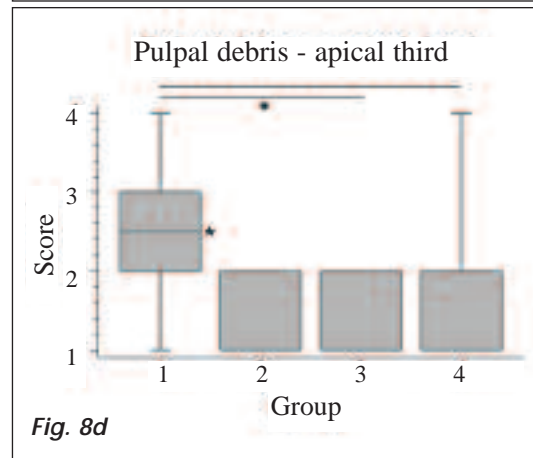
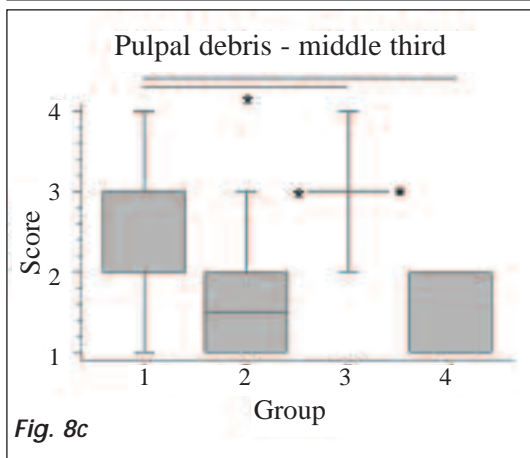
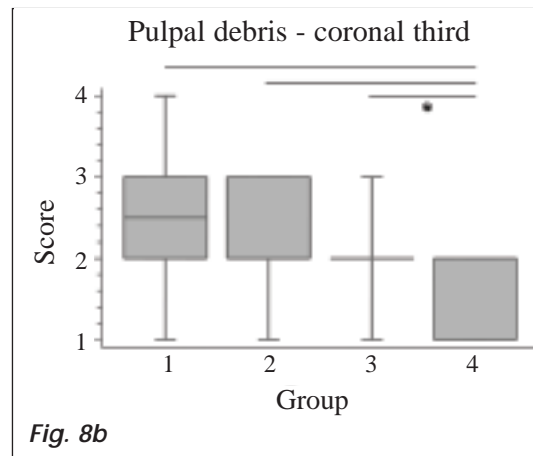
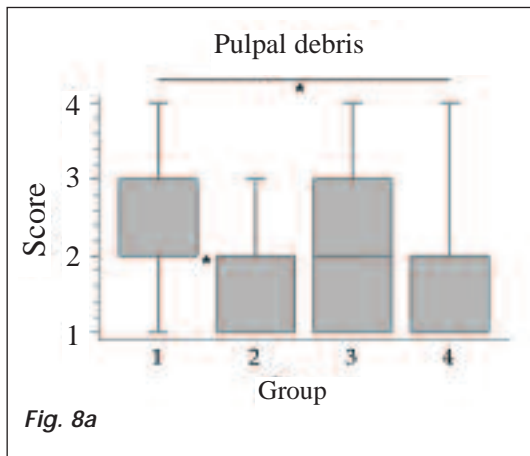
Statistical analysis

Collected scores were plotted in a Statgraphics*Plus (Manugistics, Rockville, MD, USA) spread-sheet and analyzed with the Kruskal Wallis one way ANOVA test for discontinuous ordinal values. Box and whisker plots were drawn for each parameter, showing the overall difference between the four experimental groups and specific differences between the groups for each observed third.

Results

Smear layer

Smear layer was observed in all the experimental groups (Fig. 1a). Group 4 showed the smallest presence of smear layer



Figs. 8a-b-c-d Box and whisker plots showing: a) statistically significant differences of the pulpal debris parameter between group 1 compared to group 2 and 4; b) At the coronal third in group 4 virtually no pulpal debris was observed, whereas a higher amount of pulpal debris was observed in all other groups; c) At middle third a higher amount of pulpal debris was observed in group 3; d) the apical third of specimens from group 1 presented with significantly more pulpal debris compared to the other experimental groups. *, $P < 0.05$.

compared with the other groups (1, 2 and 3) ($P < 0.05$).

While analyzing specific areas of root canal space, statistically significant differences were found in the amount of smear layer at level of the middle third (Fig. 1b). Group 1 and group 3 presented less exposed opened dentinal tubules compared to group 4 ($P < 0.05$). Group 3 (Fig. 2) was affected by a greater amount of smear layer at level of the middle third compared with group 2 (Fig. 3) and group 4 ($P < 0.05$).

Inorganic debris

Inorganic debris were identified by SEM analysis in all the experimental groups (Fig.4 a). Group 1 and group 3 presented a higher amount of inorganic debris compared to group 2 and 4 ($P < 0.05$).

At the coronal third specimens from group 3 had a significant higher quantity of inorganic debris, detectable on dentinal walls, compared to all the other groups ($P < 0.05$) (Fig. 4b). In group 2 and 4 fewer inorganic debris were left on root canal walls ($P < 0.05$). Similar findings were observed also at the middle third (Fig. 4c) (Fig. 5-7) ($P < 0.05$).

Pulpal debris

In all groups pulpal debris were observed in a variable range from a minimal collagen fibres presence to a major presence of

an organized collagenous matrix. Group 2, 3 and 4 presented a significantly less amount of pulpal debris compared with group 1 (Fig 8a) ($P < 0.05$). At the coronal third group 4 was virtually free from pulpal debris compared to the other three groups (Fig. 8b) ($P < 0.05$). Group 1 instead was affected by a collagenous matrix in different areas. At the middle third group 2 and 4 were still free from pulpal debris, while group 3 presented significantly more pulpal debris than the other experimental groups ($P < 0.05$) (Fig. 8c). At the apical third (Fig. 8d) all groups presented a low amount of pulpal debris, with the exception of group 1 (Fig. 9-10) ($P < 0.05$).

Surface profile

The surface profile of endodontic walls was homogeneous in all groups. Few pits, grooves and superficial irregularities were detected by SEM. Group 3 presented higher surface irregularities compared to the other three groups ($P < 0.05$) (Fig. 11a). At the apical third group 2 (Fig.11b) presented a homogenous surface profile (Fig.12-13).

Discussion

The purpose of this morphological root canal wall study was to compare the cleaning efficacy of four different irrigation techniques after hybrid preparation with NiTi rotary

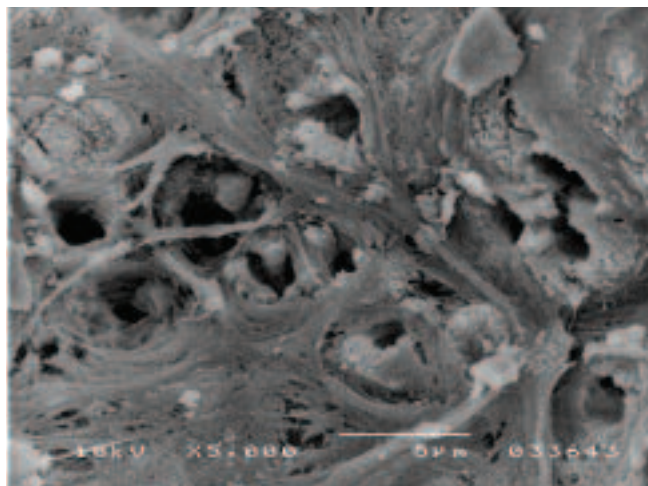


Fig. 9 At apical third the same samples presented pulp remnants with a collagenous matrix spread along the root canal walls. Group 1.

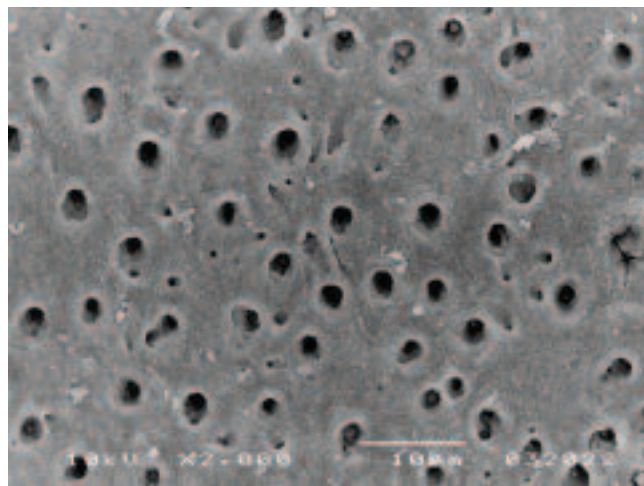
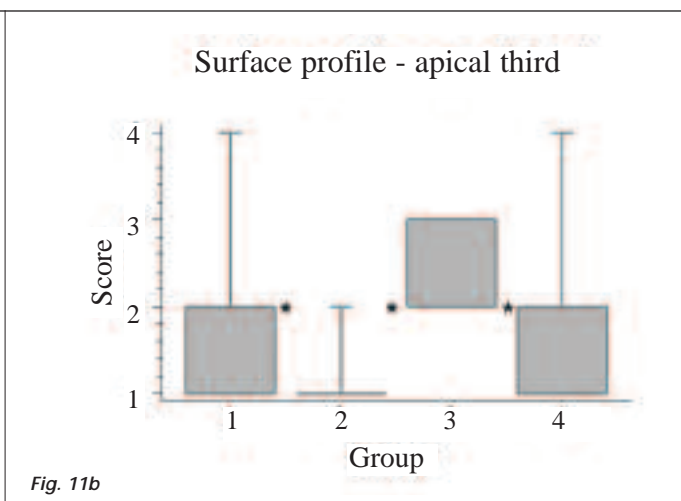
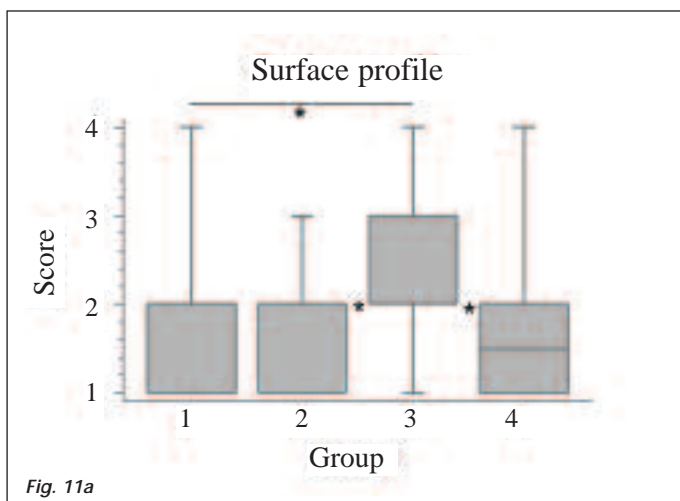


Fig. 10 Apical third of group 2.

instruments. To shape the coronal and middle portion of root canals and give them a funnel-form, ProTaper rotary instrumentation was used followed by GT and Profile rotary instruments to complete the preparation of the apical third; these canal preparation procedures were standardized in all the experimental groups. Each sample was split for flushing with different chemical solutions or their combination. As expected, group 1 canals, flushed only with NaOCl, showed inorganic debris and smear layer. This confirms that sodium hypochlorite irrigation alone is unable to remove the smear layer, as it acts mainly on the organic debris (Grandini *et al* 2002). Several in vitro studies (Cunningham & Balekjian 1980, Abou-Rass & Oglesby 1981) have shown that raising the temperature of the NaOCl improves its ability to dissolve collagen tissue. After manual root canal preparation, certain authors (Berutti & Marini 1996) found a marked difference in smear layer composition between flushing with NaOCl at room temperature (21°C) or at 50°C, noting differences in its structure and thickness as well as organization and adhesion to root canal walls. In fact, NaOCl heated to 50°C encourages

the formation of a thinner smear layer made up of finer particles, which show less adhesion to one another. Endodontic instruments may vary in their debris removal efficacy and production of smear layer, due to their blade design (Hülsmann *et al.* 2001). Irrigation therefore plays a key role in successful debridement and disinfection. The significantly lower debris and smear layer scores in groups 3 and 4 are probably due to the higher irrigating efficacy of the liquid EDTA / 5% sodium hypochlorite combination over the viscous EDTA / 5% sodium hypochlorite combination used in group 2. As reported by other authors (Peters & Barbakow 2000), in the present study, a combination of NaOCl and EDTA was found to remove both the organic tissue and inorganic smear layer; however, this did not produce the expected smear-free surface in the apical third of the canal.

This study confirms that the apical third is the area where more debris is still visible under SEM inspection. Irrespective of the rotary technique, many of the prepared canals in this study had unprepared areas in the coronal and especially the apical third. The unprepared areas of the coronal third are probably



Figs. 11a-b Box and whisker plots showing: a) statistically significant difference of the surface profile parameter between group 3 and the other three experimental groups, with a greater number of pit, grooves and surface irregularities; b) At apical third group 3 also presented a less homogenous surface profile. *, $P < 0.05$.

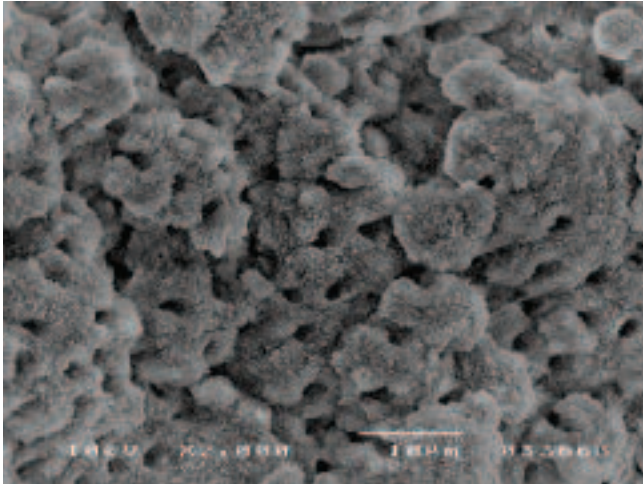


Fig. 12 Uninstrumented areas characterized by the presence of predentine were observed corresponding to grooves and depression of root canal walls. Group 3.

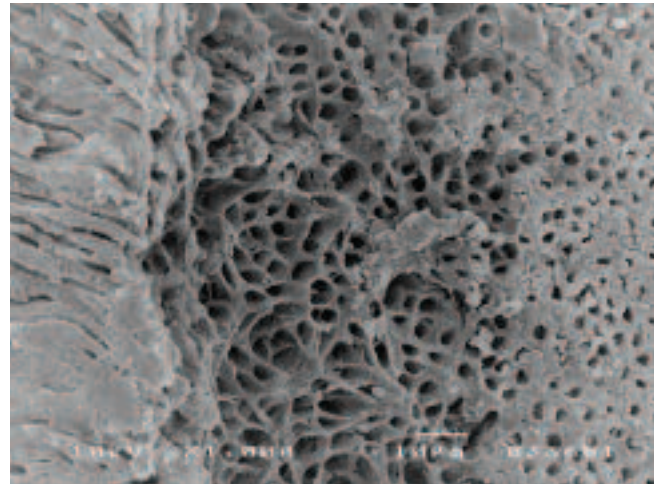


Fig. 13 "Discontinuous wave of instrumentation" : smear-free areas alternating with smear-covered areas. Group 2.

due to a general difficulty, common to all rotary techniques, in forming round preparations in many oval-shaped canals (Short *et al.* 1997). In agreement with Foschi *et al.* (2004), irregularities such as predentin, dentin grooves and depression were observed in some groups in the apical wall, and may be responsible for the presence of unprepared area. To avoid this problem Peters & Barbakow (2000) suggested larger apical stops with size 40 to 50, in order to increase the volume of irrigant reaching them.

In this study, the presence of smear-free areas alternating with smear-covered areas, a discontinuous preparation wave, in the same root dentin canal wall, may suggest that the NiTi instruments exert different pressures on the root canal wall during preparation, producing different smear layer thicknesses which are not completely removed by endodontic irrigants in the thickest parts.

Conclusion

There are limitations in this research, due to the sample size included in the study, but these findings may be used in future studies which include larger sample size to further evaluate the cleaning efficacy of root canal walls with different irrigation regimes.

In conclusion, none of the techniques used in this study enabled perfect removal of organic and inorganic root canal wall debris, although irrigation with the sodium hypochlorite / liquid EDTA combination was better than that with sodium hypochlorite and viscous EDTA. The null hypothesis tested in this study was rejected, because differences in the amount of debris along root canal walls after different irrigation regimes were noted.

On the basis of the results presented here, it is necessary to develop other procedures to achieve a dentinal canal walls smear layer free in endodontically treated teeth.

Finally, it should be emphasized that, as with most *in vitro* studies, the findings of this investigation remain to be confirmed clinically.

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