Ultrastructural analysis of the root canal walls after preparation with two rotary nickel-titanium endodontic instruments

Claudio Poggio, Alberto Dagna, Marco Chiesa, Riccardo Beltrami, Marco Colombo

Abstract

Background: Root canal preparation may produce a large quantity of smear layer that covers canal walls. **Aims:** The aim of this study was to evaluate by Scanning Electron Microscope (SEM) the root canal dentine after instrumentation with nickel-titanium rotary files, in order to evaluate the presence/absence of smear layer and the presence/absence of open tubules on the root canal walls at the coronal, middle, and apical third of each sample. **Materials and Methods:** A total of 20 single-rooted freshly extracted teeth were selected and divided into two groups. For each group, root canals were shaped with Mtwo and Revo-S instruments under irrigation with Sodium hypochlorite and 17% ethylenediaminetetraacetic acid. Specimens were fractured longitudinally and SEM analyzed at standard magnification of ×1000 and ×5000. The presence/absence of smear layer and the presence/absence of open tubules at the coronal, middle, and apical third of each canal were evaluated using a three-step scale for scores. **Statistical Analysis Used:** Numeric data were analyzed using the Kruskall-Wallis and Mann-Whitney U-Statistical tests and significance was pre-determined at $P < 0.05$. **Results and Conclusions:** This study did not reveal differences among the two groups. Mtwo and Revo-S showed no significant difference between them and both presented very low smear layer scores and open tubules scores, with no significant difference among coronal, middle, and apical third. Mtwo and Revo-S rotary instruments seem to be effective in removing smear layer from canal walls.

Keywords: Dentinal tubules, ethylenediaminetetraacetic acid, nickel-titanium instruments, smear layer

Introduction

Successful root canal treatment is based on cleaning, shaping, and sealing the root canal system.[1] The main objective of root canal therapy is the elimination of microorganisms from the root canal system and the prevention of recontamination after treatment.[2-4] Irrigating solutions are used to facilitate the debridement and disinfection of the root canal space and are considered to be essential for successful endodontic treatment.[5-8] Mechanical preparation cannot effectively eliminate bacteria from the root canal system[9] and modern rotary instrumentation techniques produce a large quantity of smear layer that covers root canal walls. In the last decade, many nickel-titanium (Ni-Ti) rotary instruments have been introduced. Several studies[10-12] demonstrated that they can efficiently create a smooth funnel-form shape with minimal risk of ledging or transporting the canals. Ni-Ti rotary instruments were introduced to improve root canal preparation[13] in association with irrigating solutions to facilitate the debridement of the canals.[9,13] Sodium hypochlorite (NaOCl) is the most commonly used irrigant. Advantages to NaOCl include the antimicrobial action, the ability of the solution to dissolve vital and necrotic tissue, the lubricating action and the mechanical flushing of debris from the canal. In addition, it is inexpensive and readily available.[1] Although NaOCl is a highly effective antimicrobial agent, it does not remove the smear layer from the dentin walls.[14-20] Ethylenediaminetetraacetic acid (EDTA) is considered a moderate antibacterial agent and it is appreciated for its ability to chelating hard tissue as decalcifying agent.[1]

The purpose of this *ex vivo* study is to investigate by Scanning Electron Microscope (SEM) image the endodontic dentinal surfaces after canal shaping with two Ni-Ti rotary instruments, under irrigation with NaOCl and EDTA, in order to evaluate the presence/absence of smear layer and the presence/absence of open tubules on the root canal walls at the coronal, middle, and apical third of each canal. The null hypothesis of the study is that there is no significant difference in debris scores and open tubules scores between the two instruments.

Material and Methods

A total of 20 single-rooted human teeth freshly extracted for periodontal reasons were selected for this study and placed in saline at room temperature immediately after extraction. The inclusion criteria were: Morphological similarity, single-canal

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roots, straight roots, and absence of root decay, absence of previous endodontic treatment, root length of at least 13 mm, and apical diameter of at least #20. The crown of each tooth was removed at the level of the cementum-enamel junction in order to obtain root segments similar 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 hand piece to facilitate vertical splitting with a chisel after canal instrumentation. All the roots were randomly assigned to two groups of 10 specimens each. The same trained operator prepared samples. The root canals were preliminary instrumented using the stainless steel #08-10-15 K-files (Maillefer, Konstanz, Germany) to create a glide path and then shaped with two Ni-Ti rotary instruments:

Group A: Mtwo (Sweden Martina, Due Carrare, Padova, Italy),
Group B: Revo-S (MicroMega, Besancon, France).

Mtwo and Revo-S are Ni-Ti rotary instruments designed for the continuous rotation. They were used with a digital endodontic engine (Endo Mate DT, NSK, Kanuma, Japan) in clockwise rotation respecting manufacturers' instructions and protocols. Mtwo protocol requires a 5 files sequence: 10/.04, 15/.05, 20/.06, 25/.06 and 30/.05. Engine was set at 300 rpm and 2.0 N/cm. Finishing preparation provides apical diameter of 0.30 mm and 5% taper. Revo-S protocol requires a three files sequence: SC1, SC2 and SU. Engine was set at 350 rpm and 3.0 N/cm. Finishing preparation provides an apical diameter of 0.25 mm and 6% taper.

Root canals were irrigated during instrumentation between each file change with 1 ml of 5.25% NaOCl followed by 1 ml of 17% EDTA. After preparation 4 ml of 17% EDTA were left *in situ* for 120 s followed by 1 ml of 5.25% NaOCl for 60 s as the final rinse. The same manufacturer (Ogna Laboratori Farmaceutici, Muggiò, Italy) prepared the endodontic irrigating solutions. The instruments always worked in the presence of the irrigating solutions, which were frequently replaced to maintain their effectiveness. Small endodontic needless (27G Kendall Monoject, Mansfield, Ma, USA) allowed to reach the apical third with the reflux of irrigating solutions. All the canals were washed with ethanol for 30 s and dried with calibrated paper points (Absorbent Paper Points, Denstply-Maillefer, Konstanz, Germany). Each sample were dipped in liquid nitrogen immediately after canal preparation and split longitudinally into two halves with a stainless steel chisel. The sections were then prepared for SEM analysis. The sections were then allowed to air-dry overnight in a desiccator at room temperature, sputter-coated with gold and prepared for SEM analysis (EVO MA 10 Carl Zeiss SMT AG, Germany).

SEM observations were obtained at standard magnification of ×5000. Six photomicrographs were taken at each third (coronal, middle, and apical). In a blind manner, three trained operators scored the presence or absence of smear layer on the surface of the root canal at the coronal, middle, and apical portion of each canal according to the following rate system developed by Rome *et al*.:[21] 0 = no smear layer, 1 = moderate smear layer, 2 = abundant smear layer. In addition, the same trained operators scored the visibility of open tubules at the coronal, middle, and apical portion of each canal according to the following criteria: 0 = all dentinal tubules opened, 1 = outlines of dentinal tubules visible or partially filled with debris, 2 = all dentinal tubules covered.

Smear layer scores and open tubules scores were calculated and statistically evaluated using Kruskall-Wallis and Mann-Whitney U-tests. Significance was predetermined at $P < 0.05$.

Results

The mean amounts of smear layer scores and open tubules scores of the various groups are reported in Tables 1 and 2. Kruskall-Wallis test showed the presence of statistically significant differences among the various groups ($P < 0.05$).

Figures 1 and 2 show representative samples of scanning electron micrographs of the root canal dentin surface of two groups.

When analyzing smear layer scores, no significant difference was reported between group A and group B ($P > 0.05$), that both showed significantly higher frequency of score "0," meaning that no particles were present. No significant differences were present in all groups among apical, middle, and coronal levels ($P > 0.05$).

Table 1: Smear layer scores

Groups	Canal level	Score=0	Score=1	Score=2	Mean	Significance
Mtwo	Coronal	9	1	0	0.1	A
	Middle	9	1	0	0.1	A
	Apical	9	1	0	0.1	A
Revo-S	Coronal	9	1	0	0.1	A
	Middle	8	1	1	0.3	A, B
	Apical	8	1	1	0.3	A, B

Table 2: Open tubules scores

Groups	Canal level	Score=0	Score=1	Score=2	Mean	Significance
Mtwo	Coronal	9	1	0	0.1	A
	Middle	9	1	0	0.1	A
	Apical	8	2	0	0.2	A
Revo-S	Coronal	8	2	0	0.2	A
	Middle	7	3	0	0.3	A, B
	Apical	7	3	0	0.3	A, B

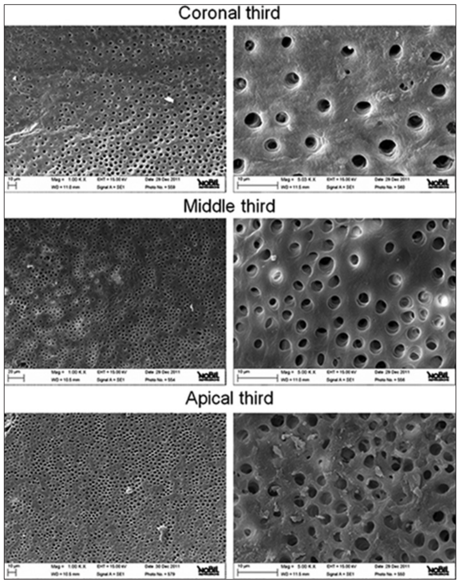

Figure 1: Representative samples of scanning electron micrographs of the root canal dentin surface instrumented with Mtwo (group A) at coronal, middle, and apical third of the root (×1000 and ×5000)

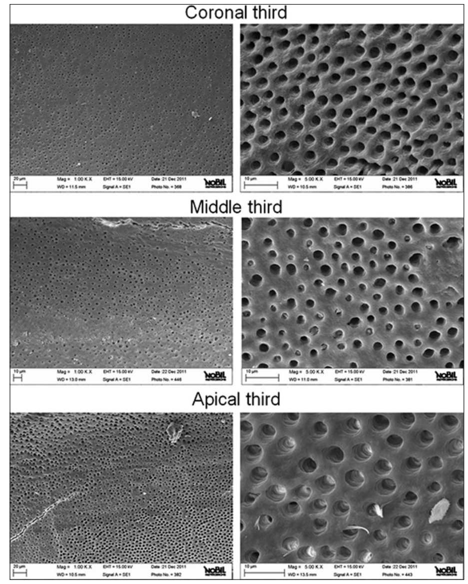

Figure 2: Representative samples of scanning electron micrographs of the root canal dentin surface instrumented with Revo-S (group A) at coronal, middle, and apical third of the root (×1000 and ×5000)

In the same way, when evaluating open tubules scores, no significant difference was reported between group A and group B ($P > 0.05$) that both showed significantly higher frequency of score "0," meaning that all dentinal tubules were open. No significant differences were present in all groups among apical, middle and coronal levels ($P > 0.05$).

Discussion

The null hypothesis of the present study has been accepted. No significant differences were found between two Ni-Ti rotary instruments.

The goal of endodontic treatment is to remove all necrotic or vital organic tissue and dentin debris created by instrumentation from the root canal system and to create root canals free from bacteria.[1] It is well-known that during root canal preparation the action of endodontic instruments produces smear layer.[2] Its elimination could allow NaOCl to penetrate more easily into the dentinal tubules; thus, enhancing its bactericidal action.[5,8] Moreover, smear layer may affect the sealing efficiency of root canal obturation, acting as a physical barrier interfering with adhesion of sealers to canal walls.[3,4] All Ni-Ti rotary instruments produced smear layer that needs to be removed with the use of irrigating solutions. The chelating agents like EDTA are currently used to remove the smear layer formed during preparation of the root canals.[22] The association of EDTA and NaOCl solutions is the gold standard in chemo-mechanical preparation of the root canals. EDTA acts upon the inorganic components of the smear layer and decalcifies the peri-and intertubular dentine and leaves the collagen exposed. Subsequently, the use of NaOCl dissolves the collagen, leaving the entrances of the dentinal tubules open.[22] For this reason an irrigation regimen similar to the methodology purposed by Foschi *et al.*[23] was used, with alternation of EDTA and NaOCl at each change of instrument.

All instruments were evaluated in accordance with the manufacturers' direction. All protocols and instruments operative sequences were respected. Irrigation procedures were standardized for all experimental groups. The same trained operator shaped all root samples.

SEM analysis revealed that Mtwo and Revo-S associated to EDTA and NaOCl irrigation leave dentine surfaces substantially free from smear layer. Despite some structural differences, modern rotating Ni-Ti instruments are able to remove the smear layer produced during instrumentation and subsequently dissolved by EDTA. Previous SEM studies investigated the effect of other Ni-Ti rotary instruments on dentine and obtained similar results.[22-25] 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 part of tubules as confirmed by Foschi *et al*.[23] The present study also confirmed that the apical third is the area where more debris is still visible under SEM inspection.[23] Rotary Ni-Ti instruments produced fine dentine particles and shavings that were spread and compacted along dentine walls and then partially dissolved by EDTA and removed coronally via flute spaces. Mtwo, thanks to their "italic S" cross-section with only two cutting edges, and Revo-S, thanks to their asymmetrical section and three cutting edges located on different radiuses, favorite debris elimination and gave SEM images generally free from smear layer, with a major part of dentinal tubules completely opened.

Conclusion

Within the limitation of this study, Mtwo and Revo-S rotary instruments seem to be effective in removing smear layer from canal walls.

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