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Penetrability of AH Plus Sealer into Dentinal Tubes after Retreatment Procedure with Rotary or Reciprocating Systems and Two Additional Cleaning Methods

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ABSTRACT

Introduction: The aim of this study was to evaluate the AH Plus sealer penetration into dentinal tubules after root canal retreatment followed by two additional cleaning methods. **Methods and Materials**: Thirty-two mandibular premolars with single canal were prepared up to the F4 ProTaper Universal instrument and filled by a single cone technique with the addition of Rhodamine B dye to Endofill sealer. For the retreatment procedure, the teeth were randomly divided into four experimental groups (n=8) as follows: Reciproc R40 with ultrasonic activation (RU), Reciproc R40 with sonic agitation (RS), ProTaper Next until X4 instrument with ultrasonic activation (PTNU), and ProTaper Next (X4) with sonic activation (PTNS). A new root canal filling was done using a System B technique, and the AH Plus sealer was mixed with Fluorescein dye. The roots were axial sectioned at 3, 5, and 7 mm from the apex and were assessed by a confocal laser scanning microscopy using the method of epifluorescence with wavelengths of absorption and emission for rhodamine B and fluorescein. In the obtained images, the sealer penetration into the dentinal tubules was evaluated. The data were converted into percentages and submitted to Mann-Whitney and Kruskal-Wallis followed by Dunn's tests (P<0.05). **Results**: In all groups were found penetration of the AH Plus into the dentinal tubules. Statistical difference was found (P<0.05) in the ProTaper Next groups in relation the Reciproc groups for 3 mm root canal level regardless of additional cleaning method used. For the other sections the sealer penetration was similar (P>0.05) for all groups. **Conclusion**: Based on this *ex vivo* study the retreatment with ProTaper Next showed significantly greater penetration of AH Plus sealer into dentinal tubules in the apical third. The additional cleaning methods did not improve the removal of filling material in all sections evaluated.

Keywords: AH Plus Sealer; Confocal Laser Scanning Microscopy; Endodontic Retreatment; Sonic Activation, Ultrassonic Activation

Introduction

Non-surgical endodontic retreatment aims to relieve patient symptoms and reestablish healthy periapical tissues following initial therapy's failure by removing gutta-percha and sealer from the root canal space, chemically disinfecting canals, and if present, addressing fails of pathological or iatrogenic origin. The complete removal of filling material from the root canal and the dentin walls'cleanness is recommended in the root canal retreatment. The removal of the root filling material from dentinal tubules seems essential to uncover bacteria that might be responsible for post-treatment disease and to eliminate them using irrigant solutions [1, 2].

Several methods and instruments are available for retreatment purposes, such as Mtwo Retreatment (VDW, Munich, Germany), ProTaper Universal Retreatment (Dentsply Maillefer, Ballaigues, Switzerland), ProTaper Next (Dentsply Maillefer, Ballaigues, Switzerland), Reciproc (VDW, Munich, Germany), among others. However, any rotary or reciprocating systems are useful to remove altogether filling material during retreatment [3-8], additional cleaning methods with sonic, ultrasonic, and laser systems have been proposed as final retreatment steps to improve the removal of the root canal filling [3, 9]. Passive ultrasonic irrigation is more effective than syringe irrigation [10] and similar to the sonic activation of irrigation solution to remove the root

canal filling [3]. The adhesion between dentine and filling materials occurs by close contact and adaptation of the materials on the root canal walls with the sealer penetration into dentinal tubules. The penetration ability of root canal filling materials into the dentinal tubules may also avoidance by residual materials on root canal walls surface [1, 11-13].

There are several types of sealers used to fill the root canals, but the AH-Plus sealer presents better behavior and has been recognized as a gold standard, considering its adaptability to the root canal walls, physical/chemical and biological properties [14-16]. Endodontic sealer should be able to promote an effective bond between the gutta-percha and the dentine walls to preventing leakage, furthermore to fill the voids inside the root canals such as the accessory canals or other inaccessible areas for core material [2]. Therefore, the removal of sealer is important in endodontic retreatment, so that the next sealer can exercise these properties in the new filling.

No study evaluated the AH-Plus sealer's dentinal tubule penetration in the re-obturation after retreatment using the Reciproc and ProTaper Next systems associated with additional cleaning methods.

Therefore, this study aimed to evaluate AH-Plus sealer' penetrability after endodontic retreatment using Reciproc and ProTaper Next systems and additional sonic and ultrasonic cleaning methods. The null hypotheses of this study are (1) there is no difference between two instruments used for AH-Plus penetration after endodontic retreatment; (2) There is no difference between penetration values of AH-Plus regardless of the additional cleaning used.

Materials and Methods

Sample size estimation

The data from a previous study [3] on retreatment using single-rooted teeth were used to determine the effect size for the present study (*ie,* 1.80). An alpha-type error of 0.05, a beta-type error 0.05 (power=0.95), and a ratio of N2/N1=1 were also stipulated. Eight specimens per group were indicated as the ideal size.

Tooth selection

Thirty-two recently extracted human mandibular premolars were selected. Inclusion criteria were a completely formed apex; a single, oval-shaped canal (buccolingual diameter twice as long as, or longer than, the mesiodistal diameter throughout the first two-thirds of the canal); and a straight root with less than 5 degree curvature according to Schneider's classification [17]. All procedures were done by a specialist in endodontics using a dental operative microscope under 12.5 magnification.

Specimen Preparation

All of the root canals were prepared using a X-Smart electric motor (Dentsply Maillefer, Ballaigues, Switzerland) to rotate the instruments of ProTaper Universal rotary system up to the F4 file (tip size: #40). The working length was determined by measuring the position of a size 10 K-file (Dentsply Maillefer, Ballaigues, Switzerland) until it could be seen through the apical foramen and then subtracting 1 mm. Foraminal patency was maintained with a #10 K-file, and 20 mL of 1% sodium hypochlorite was used as an irrigation solution for each specimen during canal shaping. After the instrumentation process the smear layer was removed using passive ultrasonic irrigation-PUI (Irrisonic Tip; Helse, Santa Rosa do Viterbo, SP, Brazil) with 1 mL of 17% EDTA for 1 minute. The Irrisonic instrument was used according to the manufacturer's instructions at a power setting of 20% of ultrasonic device. The canals were dried using F4 paper points and then were filled using the single-cone technique with gutta-percha cone F4 and Endofill sealer (Dentsply Maillefer, Ballaigues, Switzerland) mixed with Rhodamine B dye (Sigma-Aldrich, St. Louis, USA) at an approximate concentration of 0.1%. The coronal portion was sealed with temporary filling material (Coltosol; Coltene, Altstatten, Switzerland). The specimens were stored in an environment with 100% humidity at 37° C for one month.

Retreatment procedures

The 32 teeth were divided into 4 groups (n=8) according to the procedures adopted for retreatment and the additional cleaning method:

1. RU group: Reciproc R40 (tip size: #40, VDW, Munich, Germany) with PUI (Irrisonic Tip)
2. RS group: Reciproc R40 with sonic agitation (EndoActivator, Dentsply Tulsa Dental Specialties, Tulsa, OK)
3. PTNU group: ProTaper Next X2, X3, and X4 (25/06, 30/07, 40/06 respectively, Dentsply Maillefer, Ballaigues, Switzerland) with PUI (Irrisonic Tip)
4. PTNS group: ProTaper Next X2, X3, and X4 with sonic agitation (EndoActivator)

For all groups, the instruments were driven with a VDW Silver motor (VDW) in the "Reciproc All" mode up to the working length (WL) when used Reciproc R40 file and in the "Doctor's Choice" mode at the WL with a constant speed of 500 rpm and torque of 3 Ncm when used X2 (25/0.06), X3 (30/0.07) and X4 (40/0.06) ProTaper Next files. The instruments were applied in all of the groups using an "in-and-out" motion with an amplitude of 3 mm and a "brushing motion" against the lateral walls of the canal. After performing 3 strokes, the instrument was removed from the canal and

cleaned with sterile gauze, and the canal was flushed with 2.5% NaOCl. No solvent was used during all procedures. Irrigation during filling removal was performed with a total of 25 mL 2.5% NaOCl solution per tooth, and paper points were subsequently used to dry the canals. No fractures or deformations were observed.

Refilling procedures

The smear layer was removed using PUI (Irrisonic Tip, E1-Irrisonic, Helse Dental Technology, Santa Rosa de Viterbo-SP, Brazil) with 1 mL 17% EDTA for 1 min. The canals were dried using paper points, and the new root canal fillings were promoted using of Continuous Wave Condensation technique. The AH-Plus sealer (Dentsply) was manipulated according to the manufacturer's instructions and was mixed with fluorescein dye at an approximate concentration of 0.1%. In the sequence, a single master cone based in the last instrument used for retreatment was coated with sealer and inserted into the root canals until the WL. Excess material was removed using Elements Obturation Unit (SybronEndo, Orange, USA) and compacted with a hand plugger 1 mm below the canals'entrance. So, the Elements Obturation Unit was pre-set at 200 degrees Celsius, and the plugger of System B (0.06 taper) was inserted into the canal, producing a continuous wave of condensation with plugger at 4 mm of the WL (down-pack). After that, the gutta-percha was compacted with hand NiTi pluggers. The backfill procedures were performed with a 23-gauge Elements Obturation Unit needle containing gutta-percha heated at a temperature of 200 degrees driven by coronal opening. material (Coltosol, Coltene, Vigodent, Rio de Janeiro, RJ, Brazil), and the specimens were stored in an environment with 100% humidity at 37° C for 480 min.

Confocal microscope procedures

The root refilled teeth were prepared for confocal laser microscope analysis (TCS-SPE; Leica Microsystems GmbH, Mannheim, Germany). The specimens were axially sectioned using a cut machine (Extec Labcut 1010, Enfield, CT, USA) under water cooling to prevent frictional heat. It was used for sectioning the samples at 3, 5, and 7 from the apex using a 0.3 mm Isomet saw (Buehler, Lake Bluff, IL, USA) at 200 rpm. The first apical cut was discarded. The apical surface of each sample was prepared in, and living boards face up. These pieces were mounted in glass blades and polished using sandpapers number 500, 700, and 1200 under water cooling to eliminate the cutting procedure's debris product (Politriz; Arotec, Cotia, SP, Brazil) [18]. The samples were evaluated using an inverted confocal microscope (Leica TCS-SPE Leica, Mannheim, Germany) and a method of epifluorescence with wavelengths of absorption and emission rhodamine B of 540/590 nm and fluorescein of 536/617 nm. The samples were analyzed 10 µm below the surface sample by using a 10× lens and a 1 µm step size. The images were acquired at 1024×1024 pixels and were evaluated using the Image J V1.46r software (National Institutes of Health, Bethesda, USA). The total area of the root canals ware obtained first. Then, the perimeter (mm) of dental tubules in which the sealer penetrated with fluorescein were measured to determine the sealer penetration, which was expressed in percentages.

Statistical analysis

The statistical tests were Mann Whitney for comparison between the instruments independent of the irrigation method and also for comparison between the methods of irrigation independent of the instrument. The Kruskal-Wallis and Dunn test was to compare the four individualized groups. For both tests, the level of significance was set at $P \leq 0.05$.

Table 1. The median, minimum and maximum values of the percentage of dentinal penetration of the AH-Plus sealer in function of the instrument independent of the additional cleaning method and in function of the additional cleaning independent of the instruments in the different level evaluated

	Instruments		Additional cleaning	
	Reciproc	ProTaper Next	Ultrasonic	Endoactivator
3 mm	12.7 (0-41.1)[a]	38.0 (6.7-87.3)[b]	16.2 (0-78.2)[1]	20.9 (0-87.3)[1]
5 mm	17.7 (4.3-66.3)[a]	28.3 (0-67.5)[a]	23.3 (0-67.5)[1]	27.3 (10.8-64)[1]
7 mm	11 (0-43.2)[a]	22.9 (4.9-72.8)[a]	17.3 (0-70.9)[1]	21.5 (0-72.8)[1]

Different letters indicate statistical significant differences between instrument in each level and different numbers indicate statistical significant differences between additional cleaning (P<0.05)

Table 2. The median, min and max values of the percentage of dentinal penetration of AH-Plus proportioned by the studied groups in the levels analyzed

	Groups			
	Reciproc/Endoactivator	Reciproc/Ultrasonic	ProTaper Next/Endoactivator	ProTaper Next/Ultrasonic
3mm	14.4(0-41.1)[ab]	9.5(0-23.5)[b]	32.8(6.7-87.3)[a]	41.5(6.7-78.2)[a]
5mm	17.4(10.8-64)[a]	21.4(4.3-66.3)[a]	28.3(17.1-61.6)[a]	33.7(0-67.5)[a]
7mm	19.5(0-39.6)[a]	11(0-43.2)[a]	21.5(4.9-72.8)[a]	29.9(11.3-70.9)[a]

Different letters indicate statistical significant differences between the groups in each level (P<0.05)

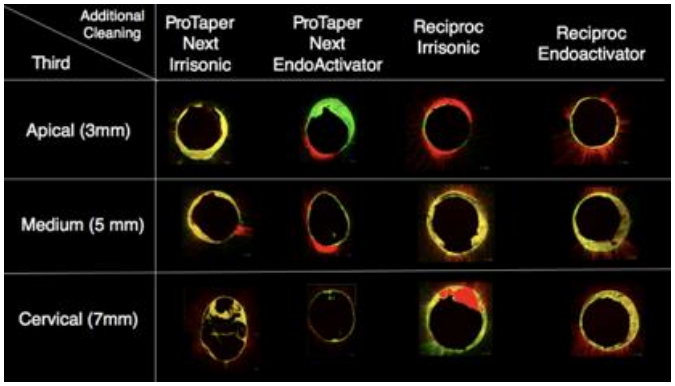

Figure 1. Representative confocal microscope images of the evaluated groups and thirds studied. Red color represents the residual sealer (Endofill sealer) from the first obturation and green color represents the AH-Plus sealer used for refilling procedures, yellow-colored indicates an overlapping of the sealers and consequently of the red and green fluorescences.

Results

Table 1 presents the median, minimum and maximum values of the percentage of dentinal penetration of the AH-Plus sealer in function of the instrument independent of the additional cleaning method and in function of the additional cleaning independent of the instruments in the different level evaluated. Statistical significant difference was found between Reciproc and ProTaper Next in the level of 3mm ($P<0.05$).

Table 2 presents the median, minimum and maximum values of the percentage of dentinal penetration of AH-Plus proportioned by the studied groups in the levels analyzed. Statistical significant differences occurred in the comparisons of the Reciproc/PUI group with the ProTaper Next/Endoactivator and ProTaper Next/PUI groups ($P<0.05$)

The Figure 1 presents the confocal representative images of the each studied group in the different levels.

Discussion

Confocal laser scanning microscopy (CLSM) allows the detection of sealer penetration through the dentinal tubules and has significant importance in endodontic retreatment and additional irrigation evaluation.

The root canal retreatment aims to completely remove the filling material, which consists of a physical barrier that blocks or decreases the new attempt to disinfect the root canal system. Many endodontic instruments were used to perform the retreatment procedure, among then rotary and reciprocating NiTi instruments have shown very useful for this purpose [3]. Reciprocate instruments have been used for endodontic retreatment with satisfactory results and can be proven by several articles in the literature [5-7].

Among the various rotary systems, ProTaper Next can be used for endodontic retreatment [3, 8, 19], but the methods used for additional cleaning, include sonic or ultrasonic methods, equivalents with the micro-computed tomographic study [3].

Unirradiculated teeth were used to have a more favorable sealing condition, and consequently to seal, to evaluate the penetration capacity of the sealant cement in a more controlled environment, excluding the bias that the anatomical complexity can impose. After obturation, the specimens were stored at 37ºC and 100% humidity for one month to ensure the sealer was fully set. To achieve a degree of uniformity and reduce inter-operator variables, the same operator conducted all procedures.

The dentinal tubule penetration area was significantly affected by the selection of ProTaper Next instruments in the apical third regardless of the additional cleaning methods.

The first null hypothesis was not accepted because when used ProTaper Next there was more penetration of AH Plus sealer after retreatment procedures the apical third. The second null hypothesis was accepted because there wasn't statistical difference about when used ultrasonic and sonic irrigation concerning the penetration of AH-Plus Sealer.

Considering the endodontic sealers' chemical diversity, this study assessed the impact of remaining zinc oxide-eugenol–based sealer on the adhesion of a resin-based sealer to dentine after root canal retreatment using Reciproc 40 or Protaper Next X2, X3, and X4, and additional cleaning methods. The phenolic compounds of eugenol are free radicals that diffuse into dentine and hamper the polymerization of resinous materials [20]. The free radicals also modify the polymerized resin's surface, thus reducing the efficacy of adhesion [21, 22]. The apical third can have better penetration for AH Plus because ProTaper Next showed a better ability to filling removal, probably related to their specific design with a rectangular cross-section and snack movement. Its design allows the instrument to touch the wall in only two places, making it a larger area of the escape of the sealer material. Despite both instruments have the same tip and taper, probably the design of the instrument possibility better sealer penetration in the apical third (3mm). Both instruments present similar ability to promote microcracks [23].

There is no significant statistical difference after the additional cleaning of the root canals using sonic (Endoactivator) or ultrasonic (Irrisonic) irrigation when observed the three thirds. Another study observed that the ability to remove smear layer of the ultrasonic and sonic activations were similar [24]. The large diameters and the higher number of dentinal tubules in the middle and cervical thirds facilitated the new filling material's penetration into tubules.

The additional irrigation methods improve the sealing cement penetration, as was also observed in other studies [25, 26], where there was a comparison between some additional irrigation methods and the syringe and needles alone, and there was no adequate cement penetration in the endodontic treatment. In our study, the endodontic retreatments were performed, and it was observed that even using additional cleaning methods, there was not a significant penetration of cement in general. Concerning the use of the instruments to remove the filling material, the ProTaper Next favored a higher penetration of the AH-Plus in the dentinal tubule at the apical third.

The presence of a yellow-colored indicates an overlapping of the sealers and consequently of the red and green fluorescence. The yellow color was considered as the penetration of AH-Plus sealer.

This study didn't use solvents in retreatment procedures, in contrast with another research [1, 27], where the adhesion of AH-Plus to dentine walls was higher in the group that combined ultrasound with xylol. Probably if our study had used solvent, the results could be different with better outcomes for AH-Plus penetration into the dentinal tubules.

In contrast, another study [3] showed that the deep and percentage of sealer penetration is influenced by the type of insertion technique and by the root canal level, with penetration decreasing apically. This study used different techniques to insert sealer into the root canals. In the first treatment lentulo spirals were used, and in the re-obturation just gutta-percha cone with sealer.

The penetration of AH-Plus sealer into dentinal tubules was limited in medium and cervical thirds, because the Endofill sealer used in the initial filling presented high penetration in the referred regions. According our results, another study observed higher penetration ability of the AH-Plus in medium and cervical thirds [28]. In endodontic retreatment, the interaction between zinc oxide-eugenol-based sealer and resin-based sealer may not result in favorable bond strength of filling material to dentine [1].

Conclusion

Within the limitation of this study, the retreatment with ProTaper Next showed significantly higher penetration of AH-Plus sealer into dentinal tubules in the apical third regardless of additional cleaning used. The additional cleanings methods were equivalent and did not improve the removal of filling material in all thirds studied.

Conflict of Interest: 'None declared'.

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