Penetrability of AH Plus and MTA Fillapex After Endodontic Treatment and Retreatment: A Confocal Laser Scanning Microscopy Study

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ABSTRACT The aim of the study was to assess the penetrability of two endodontic sealers (AH Plus and MTA Fillapex) into dentinal tubules, submitted to endodontic treatment and subsequently to endodontic retreatment. Thirty ex vivo incisors were prepared using ProTaper rotary system up to F3 instrument and divided in three groups according to the endodontic sealer used for root canal filling: AH Plus (AHP), MTA Fillapex (MTAF), and control group (CG) without using EDTA previously to the root canal filling. Rhodamine B dye (red) was incorporated to the sealers in order to provide the fluorescence which will enable confocal laser scanning microscopy (CLSM) assessment. All specimens were filled with gutta-percha cones using the lateral compaction technique. The specimens were submitted to endodontic retreatment using ProTaper Retreatment system, re-prepared up to F5 instruments and filled with gutta-percha cones and the same sealer used during endodontic retreatment. Fluorescein dye (green) was incorporated to the sealer in order to distinguish from the first filling. The roots were sectioned 2 mm from the apex and assessed by CLSM. No difference was found between the two experimental groups ($P > 0.05$). On the other hand, in the control group the sealers were not capable to penetrate into dentinal tubules after endodontic treatment ($P > 0.05$). In retreatment cases, none of the sealers were able to penetrate into dentin tubules. It can be concluded that sealer penetrability is high during endodontic treatment. However, MTA Fillapex and AH Plus do not penetrate into dentinal tubules after endodontic retreatment. *Microsc. Res. Tech. 77:467–471, 2014*.

INTRODUCTION

Endodontic retreatment is a procedure performed in a tooth that has already received a previous attempt of treatment. However, this attempt resulted in a condition that requires new endodontic intervention to achieve the clinical and radiographic success (Bueno et al., 2006). Among several treatment alternatives, nonsurgical retreatment should be considered as the first choice (Hulsmann et al., 2004).

The removal of gutta-percha and endodontic sealer followed by reinstrumentation of root canal provides better removal of the necrotic tissues and microorganisms that may be responsible for the persistent periapical inflammation. However, complete root canal cleaning is almost impossible, regardless of the technique used for root canal preparation (Só et al., 2008).

MTA Fillapex and AH Plus are classified as resin based sealers, presenting different compositions. AH Plus, an epoxy resin based sealer, has been considered the gold standard for its physic and chemical properties, such as radiopacity, biocompatibility, flow, and sealing (Flores et al., 2011). MTA Fillapex, a salicylate resin based sealer containing MTA, is biocompatible, and presents adequate working time, easy handling and acceptable radiopacity (Rosa et al., 2013) MTA Fillapex also presents little antibacterial activity before setting (Morgental et al., 2011).

The aim of the study was to analyze the penetrability of AH Plus and MTA Fillapex into dentinal tubules, submitted to endodontic treatment and subsequently to endodontic retreatment. The null hypotheses of this study are: (1) there is no difference between sealer penetration after endodontic treatment and retreatment; (2) there is no difference between penetration values of AH Plus and MTA Fillapex regardless if they were used for endodontic treatment or retreatment.

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MATERIALS AND METHODS

Sample Size Calculation and Sample Selection

The sample size was calculated using the BioEstat 4.0 statistical software (Belém, PA, Brazil) with a statistical power of the study of 80%. Thirty ex vivo maxillary incisors were selected from a tooth bank after project approval by the ethical committee of the Federal University of Rio Grande do Sul. Only teeth presenting complete root formation, absence of external and internal root resorption, without calcifications and previous endodontic treatment were included in this study. Specimens were stored in 0.9% saline solution until use.

Root Canal Preparation

Specimens were sectioned at the enamel cement junction, using double-sided diamond blades, and standardized in a length of 12 mm. The glide path was verified with a #10 K-file (Dentsply-Maillefer, Ballaigues, Switzerland) until the tip of the instrument was just visible at the apical foramen. Then the silicon stop was positioned at the reference point and the measure was recorded. The working length (WL) was established as being 1 mm shorter than this measure. Root canal preparation was performed by the same operator (D. K.) using ProTaper Universal instruments (Dentsply-Maillefer, Ballaigues, Switzerland) with an X-Smart motor (Dentsply-Maillefer, Ballaigues, Switzerland) at 250 rpm and a torque setting of up to 2.5 N/cm.

Initially, the cervical thirds were pre-flared using the SX instrument and S1 to two-thirds of the estimated WL. Next, rotary instrumentation was accomplished using S1, S2, F1, F2, and F3 to full WL. Between each file change, the root canals were rinsed with 2 mL of 2.5% sodium hypochlorite (NaOCl), delivered with a syringe and 30-gauge needle (NaviTip, Ultradent, South Jordan, UT) inserted to 2 mm short of the entire working length.

Samples were randomly divided into two experimental groups and a control group, according to the endodontic sealer used for root canal filling: AHP, AH Plus (Dentsply-Maillefer, Ballaigues, Switzerland) (N = 10); MTAF, MTA Fillapex (Angelus, Londrina, PR, Brazil) (N = 10); CG, control group (N = 10) subdivided into AH Plus (N = 5), and MTA Fillapex (N = 5). After the completion of preparation, the roots from experimentally groups were irrigated with a final sequence of 5 mL of 17% EDTA for 3 min and 2 mL of 2.5% NaOCl. Next, the canals were dried using F5 absorbent paper points. The roots from control group were not flushed with EDTA for smear layer removal.

Endodontic Treatment: Root Canal Filling

Root canal filling was performed by a single operator (D. K.), previously trained, using the lateral compaction technique and F3 and FM gutta-percha cones (Dentsply-Maillefer, Ballaigues, Switzerland). Root canal sealers were mixed according to the manufacturer's specifications. Rhodamine B dye in a ratio of 0.1% (Alpine et al., 2006) was added to root canal sealers, in order to provide the fluorescence which will enable CLSM assessment. Samples were stored for 2 weeks at 100% moisture and 37°C.

Endodontic Retreatment: Preparation and Root Canal Filling

Specimens were submitted to endodontic retreatment by using ProTaper Retreatment instruments (Dentsply-Maillefer, Ballaigues, Switzerland). These instruments were used at 500 rpm and a torque setting of up to 2 N/cm, to remove root fillings by using D1, D2, and D3 retreatment instruments. D1, D2, and D3 have a taper/tip diameter of 0.09/0.30, 0.08/0.25, and 0.07/0.20 mm, respectively. As recommended by the manufacturer, D1 instrument was used at the cervical canal third, D2 at cervical and middle thirds and D3 in the full WL. All instruments were used with brushing movements against the canal walls in a crown down manner.

Then, root canals were reprepared using the instruments F4 and F5 of the ProTaper Universal system as described before. The irrigation procedures were maintained equal to the endodontic treatment. Canals were irrigated with 2 mL of 2.5% sodium hypochlorite after each instrument change. The final protocol was performed with 5 mL of 17% EDTA during 3 min and final rinse with 2 mL of 2.5% NaOCl. The roots from control group were not flushed with EDTA for smear layer removal.

Fluorescein dye (green) was incorporated to the sealers in a ratio of 0.1%, in order to distinguish from the first filling (red). The filling procedures were identical to those performed during endodontic treatment. Samples were stored under 100% moisture and 37°C for 2 weeks.

Sample Preparation for CLSM Assessment

A cut machine (Extec Labcut 1010, Enfield, CT) was used for sectioning transversally the roots at 4 mm from the apex. For CLSM assessment, a 2-mm slice was obtained. Thereafter, surfaces were polished with Arotec paste (Arotec, Cotia, SP, Brazil) for eliminate dentin debris eventually generated during the cut procedures.

The coronal surface of the samples was examined with the Olympus FluoView Confocal Laser 1000 Microscope (Olympus Corporation, Tokyo, Japan). The respective absorption and emission wavelengths for rhodamine B and fluorescein were 540/590 nm and 494/518 nm, respectively. Dentin samples were analyzed using the ×10 Zoom 3 oil lens.

Analysis of the images

The sealer penetration area within the dentinal tubules was measured by Adobe Photoshop CS6 (Adobe Systems, San Jose, USA). Initially, the total amount of pixels in the image was recorded. Then, the "lasso" tool delimited the root canal area and such value (in pixels) was also recorded. By subtracting the total amount of pixels and the pixels from the main root canal, the area of dentin that could be impregnated by the sealers was obtained. Next, the dentin area in which the sealer penetrated was enclosed by the "magic wand" tool. The area stained with red (rhodamine) displays the sealer penetration during endodontic treatment. The area of dentin penetrable corresponded to 100% and the stained area was calculated proportionally. The same procedure was

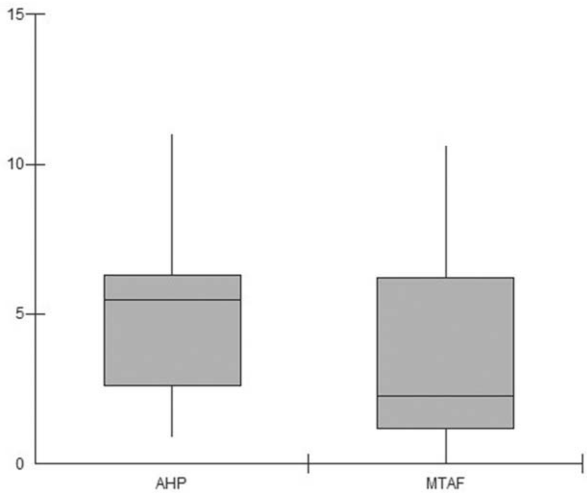

Fig. 1. Median, quartiles, and maximum and minimum values of sealer penetrability (percentage) in each group after endodontic treatment.

performed with the area stained in green (fluorescein), corresponding to the endodontic sealer that penetrated into the dentinal tubules during retreatment.

Statistical Analysis

Statistical analysis was performed using Kruskal-Wallis and Dunn's post-hoc tests to compare the sealers and the control group in each endodontic stage (treatment and retreatment). Friedmann test was used to compare the same sealer in two different experimental times (i.e., treatment and retreatment). Significance level was set at 5%.

RESULTS

After endodontic treatment, both MTAF and AHP groups presented sealer penetration within the dentinal tubules. The Kruskal-Wallis test indicated differences among the groups ($P < 0.05$). The Dunn's post hoc test showed no differences between the two experimental groups ($P > 0.05$); however, AHP and MTAF presented more tubule penetration than CG ($P < 0.05$). The median of percentage of tubule penetration of AHP and MTAF groups were 5.4% and 2.3%, respectively. Figure 1 presents the median, quartiles, and maximum and minimum values of tubule penetration in the three groups after endodontic treatment. In retreatment cases, none of sealers was able to penetrate into dentin tubules. Friedmann test showed that the area of sealer penetration within the dentinal tubules was higher in cases of treatment when compared with cases of endodontic retreatment ($P < 0.05$).

DISCUSSION

Epidemiological studies have shown, through radiographic and CT images, high incidences of periapical infections associated with pre-existing endodontic treatments (Chen et al., 2007; De Moor et al., 2000). To increase the success rates of endodontic retreatments it is required the effective removal of root filling material to provide better access of irrigants and canal dressings through root canal system. This way, the ability of disinfection obtained by chemo-mechanical preparation can be optimized (Sae-Lim et al., 2000).

Several techniques have been described for gutta-percha removal—including rotary instruments, hand files, solvents, and their associations (Cavenago et al., 2014; Friedman et al., 1990). In this study, ProTaper Retreatment system was used due its effectiveness in removing filling material (Duarte et al., 2010; Só et al., 2008; Takahashi et al., 2009). In addition, rotary instruments seems to provide shorter time for filling removal (Ferreira et al., 2001; Giuliani et al., 2008; Sae-Lim et al., 2000). Rotary instruments results in easier penetration into the filling which will facilitate the gutta-percha and sealer removal (Rödig et al., 2012). The use of solvents could not improve filling removal because it results in a soft mass of filling material that adheres to root canal walls and isthmus (Takahashi et al., 2009; Xu et al., 2012). However, several studies have shown that regardless of the technique, type of instrument (i.e., manual or rotary system) and the use of solvents, it is impossible to completely remove filling material from root canal walls and isthmus (Gu et al., 2008; Xu et al., 2012).

ProTaper Universal system includes shaping instrument (S), finishing files (F), and instruments for filling removal during retreatment (D1, D2, D3). The D1 instrument has an active tip which facilitates the initial gutta-percha penetration (Gu et al., 2008). Although rotary instruments promote faster filling removal, its use should be cautious, since the amount of dentin removed is greater when compared with manual instruments (Rödig et al., 2012). Excessive dentin removal should be avoided to minimize root weakening and the risk of radicular fractures or perforations.

In this study, two resin-based sealers were used to evaluate the penetration within the dentinal tubules during treatment and endodontic retreatment. On the basis of the results obtained, the null hypotheses were rejected. In specimens from MTAF group, 2.3% of the dentin was impregnated by the sealer, while in AHP group the sealer penetrated in 5.4% of the dentin after endodontic treatment ($P > 0.05$). The similarity of the results presented by both sealers can be explained because the resin nature of these sealers. Although MTA Fillapex is constantly called as MTA-based sealer, its composition is predominantly resinous, especially salicylate resin (Rosa et al., 2013). Up to this moment, there is no study that evaluated the penetrability of MTA Fillapex within the dentinal tubules. Silva et al. (2013) compared MTA Fillapex and AH Plus flow in accordance with ISO standards. The authors observed greater flow of Fillapex MTA (31 mm) than AH Plus (26 mm). However, this difference was not confirmed when sealers were inserted in root canals of human teeth.

Lateral compaction technique was used because it is widely performed in clinical practice. Additionally, Kok et al. (2012) demonstrated that the filling technique does not influence the penetration of resin sealers within dentinal tubules.

Preliminary studies have not confirmed the relationship between the sealing ability of endodontic sealers and their penetration in dentinal tubules (De Deus et al., 2012). In fact, sealer penetration assumes

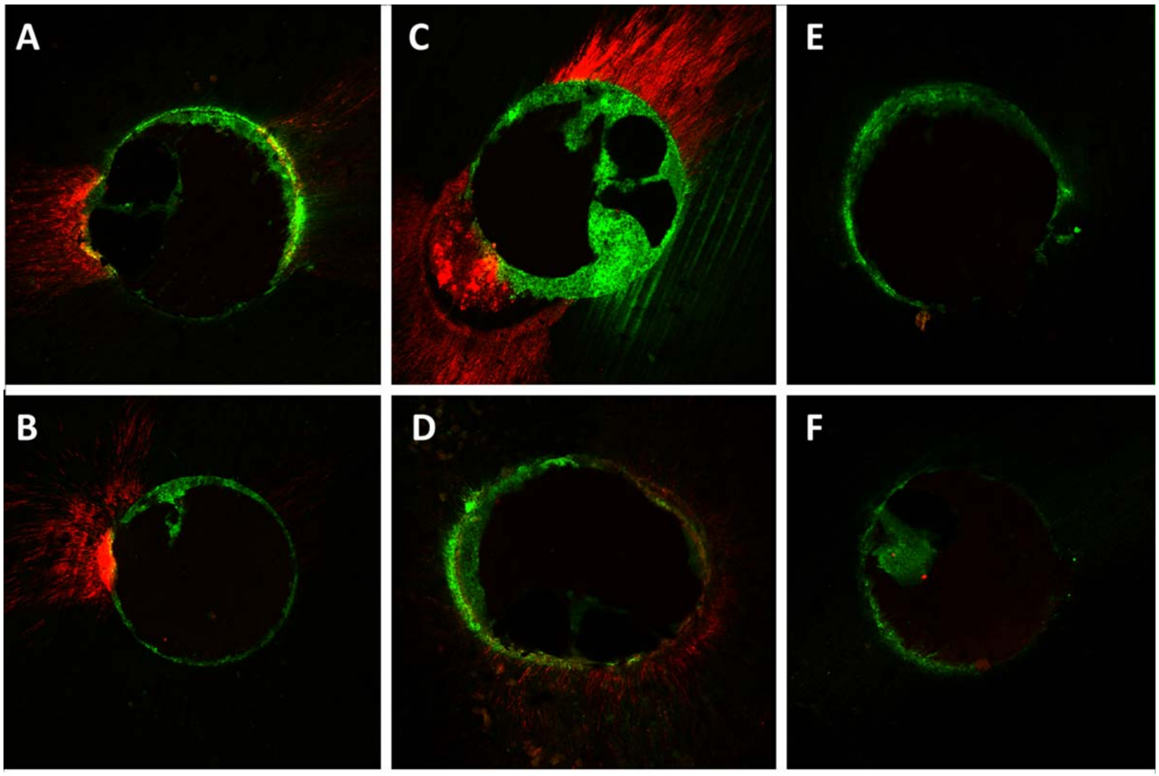

Fig. 2. Confocal laser scanning microscopy (×10) of the MTA Fillapex Group (A,B), AH Plus (C,D) and the control group (E,F). Red: sealer stained with Rhodamina (treatment); Green: sealer stained with Fluorescein (retreatment). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

importance, since endodontic sealers, unlike gutta-percha, are able to penetrate in dentinal tubules, isthmus, and accessory canals. In theory, sealer penetration in dentinal tubules would provide entombing of microorganisms in these areas, far away from nutrition (Sundqvist and Figdor, 2003). The viability of these microorganisms could be responsible for the maintenance and progression of periapical infection (Ordinola-Zapata et al., 2010).

Despite of the similar penetration of endodontic sealer observed in this study, it was not uniformly throughout the perimeter of root canal. It is possible to observe that sealers were able to penetrate in certain dentin areas, while in other portions it did not occur. This variation could be explained by the fact that dentin layer formed during the chemo-mechanical preparation has not been completely removed. Chopra et al. (2012) and Castagna et al. (2013) presented the difficulty to complete removal of debris and smear layer after root canal preparation, especially in the apical third, regardless of the protocol used. In addition, the sealer penetration in certain dentine areas may have been favored by the existence of areas where the instrument did not reach all root canal surface, allowing sealer access to dentin tubules. However, the control group, in which EDTA was not used for smear layer removal, confirmed that the presence of smear layer prevents the sealer penetration. No specimen from control group presented sealer penetration within dentinal tubules. The CG also confirmed that smear layer was formed during the root canal preparation and repreparation.

In the control group, which did not use EDTA, sealer penetration was not observed. This negative influence of smear layer was also demonstrated in the study of Tuncer et al. (2012), which evaluated the tubular penetration of AH 26 sealer after different irrigating protocols. The authors demonstrated that sealer penetration achieved high percentages values when chelating solutions were used (i.e., citric acid, maleic acid, and EDTA). The CG in which canals were irrigated only with NaOCl, showed a significantly lower percentage of sealer penetration than the other groups in all root canal thirds.

To distinguish the sealer used in initial treatment with the sealer used on retreatment it was necessary the use of two fluorescent dyes, Rhodamine (red), during endodontic treatment, and Fluorescein (green), in retreatment. Such dyes allowed the formation of high-contrast points to show the sealer distribution within the dentinal tubules by CLSM (Gharib et al., 2007; Ordinola-Zapata et al., 2008). One of the advantages of confocal laser scanning microscopy compared to scanning electron microscopy (SEM) is that the CLSM does not require sample preparation techniques that could cause artifacts (Van Meerbeek et. al., 2000). Another important advantage of this method is that the same samples were used for the treatment and subsequent retreatment. This aspect eliminates biases such as anatomical issues that may influence the results.

After filling removal, reinstrumentation, and filling, the endodontic sealers were unable to penetrate into dentin tubules (Fig. 2). The absence of sealer penetration in endodontic retreatment cases may be justified by the presence of the sealer in the primary endodontic treatment. According to Só et al. (2012), ProTaper Retreatment system were not capable of completely remove gutta-percha and endodontic sealer from palatine canals of maxillary molars. These results are similar with the findings of Duarte et al. (2010). These authors observed that all specimens presented residual filling material, especially in the apical portion. For this reason, in this study, the apical third was chosen to assess the dentinal penetration of AH Plus and MTA Fillapex (Duarte et al., 2010; Gu et al., 2008; Só et al., 2008).

CONCLUSIONS

On the basis of the methodology and results it can be concluded that: (1) the penetrability of endodontic sealers depends of the stage of the endodontic treatment (i.e., treatment or retreatment); (2) EDTA is essential to remove smear layer and to promote sealer penetration within the dentinal tubules; (3) AHP and MTAF groups presented similar tubule penetration after endodontic treatment; and (4) endodontic sealers were unable to penetrate into dentinal tubules after endodontic retreatment.

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