Microscopic Analysis of the Quality of Obturation and Physical Properties of MTA Fillapex

PABLO ANDRÉS AMOROSO-SILVA,* BRUNO MARTINI GUIMARÃES, MARINA ANGÉLICA MARCIANO, MARCO ANTONIO HUNGARO DUARTE, BRUNO CAVALINI CAVENAGO, RONALD ORDINOLA-ZAPATA, MARCELA MILANEZI DE ALMEIDA, AND IVALDO GOMES DE MORAES
Department of Operative Dentistry, Endodontics and Dental Materials, Bauru School of Dentistry, University of São Paulo, Bauru - São Paulo, Brazil

KEY WORDS MTA Fillapex; physical properties; confocal microscopy

ABSTRACT This study analyzed the quality of obturation and physical properties of MTA Fillapex and AH Plus sealer. A sample of 30 human maxillary central incisors were instrumented with Protaper until a F5 (50/05) file. Both sealers were mixed with Rhodamine-B dye to allow visualization on a confocal laser-scanning microscope (CLSM). Next, the canals were filled using the single cone technique. After setting, all samples were sectioned at 2, 4, and 6 mm from the apex. CLSM was used to analyze the gaps and sealer penetration into the dentinal tubules. All samples were scanned 10 μm below the dentin surface and images were recorded at 100× magnification using the fluorescent mode. Additionally, the solubility, flowability and setting time of the sealers were evaluated. All the measured quantities of the examined materials were evaluated for significant differences by means of statistical analysis. The CLSM analysis of the MTA Fillapex showed the highest percentage of gaps at all sections (P = 0.0001). Physical tests revealed adequate properties for both sealers except for a higher solubility of the MTA Fillapex (P = 0.0001). The MTA Fillapex presented flowability and intratubular penetration similar to the AH Plus. Nevertheless, the MTA Fillapex sealer presented a higher solubility and considerable quantity of gaps between the sealer/dentin interface in relation to the AH Plus sealer. Clinicians must take into consideration, the quality of endodontic sealers as it is essential in the outcome of the root canal filling. *Microsc. Res. Tech. 77:1031–1036, 2014.* © 2014 Wiley Periodicals, Inc.

INTRODUCTION

Some of the objectives of root canal obturation are to adequately fill the root canal system; entomb remaining bacteria; and favor periapical healing (Hargreaves et al., 2011). Endodontic sealers present an important role in achieving these objectives. They fill the spaces that cannot be reached by the gutta-percha and provide a bonding effect between the materials and dentin surface (Gatewood, 2007). In addition, an adequate penetration of the sealer inside the dentinal tubules and its adaptation to the dentinal walls can prevent leakage, avoiding the entrance of inflammatory exudate, bacteria, saliva, and chemical fluids to the interior of the canal (Ersahan and Aydin, 2013). Certain important factors may interfere with the sealer adhesion, such as the root canal instrumentation and cleaning, the filling technique, and the type of sealer (Ricucci et al., 2009).

In endodontics, different types of sealers are regularly used and their physical/chemical and biological properties should meet the criteria described by Grossman (1988) which include: an excellent seal when set, dimensional stability, a slow setting time to ensure sufficient working time, insolubility to tissue fluids, adequate adhesion with the canal walls, and biocompatibility. These factors have a direct influence on the quality of the final obturation. The American National Standards Institute/American Dental Association (ANSI/ADA) specification for endodontic sealers recommend that sealers should present a solubility lower than 3%, flow with ≥20 mm of disc diameter, and a setting time that does not exceed 10% of the time specified by the manufacturer (ANSI/ADA's specifications 57).

In recent years, the MTA Fillapex has been introduced and its physical–chemical and biological properties have been recently studied. Nevertheless, the results of these studies addressing these properties are controversial. Some authors reported higher values of solubility for MTA Fillapex (Borges et al., 2012; Faria-Junior et al., 2013; Viapiana et al., 2013), while others reported lower values (Vitti et al., 2013; Zhou et al., 2013). The biocompatibilty test has also been found to be variable (Zmener et al., 2012; Tavares et al., 2013). However, to date there is a lack of studies that evaluate the filling quality of MTA fillapex.

Thus, the aim of this study was to evaluate the quality of adaptation to the dentinal walls, sealer penetration, and physical properties of the MTA Fillapex sealer. The epoxy resin AH Plus sealer was used for comparison based on its good properties and adaptability to the root

*Correspondence to: Pablo Andrés Amoroso Silva; Faculdade de Odontologia de Bauru – USP, Al. Octávio Pinheiro Brisolla, 9-75, CEP 17012-901, Bauru, São Paulo, Brazil. E-mail: pabloamoroso@usp.br

Received 12 May 2014; accepted in revised form 19 August 2014
REVIEW EDITOR: Prof. Alberto Diaspro
DOI 10.1002/jemt.22432
Published online 10 September 2014 in Wiley Online Library (wileyonlinelibrary.com).

canal walls (De-Deus et al., 2011; Marciano et al., 2011). The hypothesis tested is that there is no difference in the filling quality and physical properties of a MTA Fillapex sealer and an epoxy resin based sealer (AH Plus).

MATERIAL AND METHODS

Sample Preparation

Thirty human maxillary central incisors with straight root canals were selected (ECP 130/2011). Next, the crowns were removed using a diamond saw at 200 rpm (Isomet, Buehler, Lake Bluff, IL, USA) leaving 15 mm of root length. The working length was established with a #10-K file at 1 mm from the apical foramen. Root canal shaping was performed using the ProTaper system (Dentsply, Maillefer, Ballaigues, Swisterland) until a F5 (50.05) instrument. A volume of 1mL of 2.5% sodium hypochlorite (NaOCl) was used to irrigate the canal after the use of each instrument. After shaping, the canals were irrigated with 3 mL of 2.5% NaOCl, 17% EDTA (Biodinâmica, Ibiporã, Paraná, Brazil) for 3 minutes and washed with 3 mL of distilled water. Finally, the canals were dried with paper points and the specimens were randomly divided into two groups ($n = 15$) with the following sealers:

- AH Plus, Lot 1107001111, (Dentsply Maillefer, Konstanz, Germany) composed of bisphenol-A epoxy resin, bisphenol-F epoxy resin, calcium tungstate, zirconium oxide, silica, iron oxide pigments, dibenzyldiamine, aminoadamantane, tricyclodecane-diamine, calcium tungstate, zirconium oxide, and silica silicone oil.
- MTA Fillapex, Lot 18535 (Angelus, Londrina, Paraná, Brazil) composed of salicylate resin, diluting resin, natural resin, bismuth trioxide, nanoparticulated silica, MTA, and pigments.

The sealers were mixed according to the manufacturer's instructions. To allow visualization under a confocal laser-scanning microscope all sealers were mixed with fluorescent rhodamine-B dye (Sigma-Aldrich, St Louis, USA) at 0.1% concentration according to previous studies (D'Alpino et al., 2006; Marciano et al., 2011). The sealers were inserted in the root canals using a size 40 lentulo spiral. Then, a tapered F5 gutta-percha cone coated with sealer was inserted until the working length and seared off with a heated instrument. The cervical portion of the specimens was sealed using a provisional filling material (Coltosol, Coltene, Altstatten, Switzerland). Next, the specimens were stored at 37°C and 100% humidity for 7 days.

Confocal Analysis

The samples were placed in epoxy resin (Triepox; Socorro, São Paulo, Brazil) and sectioned horizontally at 2, 4, and 6 mm from the apex using the diamond saw at 200 rpm with continuous water cooling to prevent frictional heat, resulting in a total of 90 slices. The polishing of the samples was performed using a Politriz machine (Arotec, Cotia, São Paulo, Brazil). The interfacial adaptation (gaps) and sealer penetration into the dentinal tubules were analyzed on an inverted Leica TCS-SPE confocal laser-scanning microscope (CLSM) using a 10× objective (Leica Microsystems GmbH, Mannheim, Germany). The absorption and emission wavelengths for the rhodamine-B were set to 540 and 590 nm. All samples were scanned 10 μm below the dentin surface and the images were recorded using the fluorescent mode to a size of 1024 × 1024 pixels.

The quality of the sealer/dentin interface was evaluated by calculating the ratio of the circumference of the canal (root canal perimeter) and then the gap-containing regions (Marciano et al., 2011). Also, to obtain the percentage of sealer penetration the circumference of the root canal was measured and the sections along the canal wall in which the sealer penetrated into the dentinal tubules were outlined and measured. Both results were expressed in terms of percentage. Both measurements were performed twice to ensure reproducibility using the ImageJ V1.46r software (US National Institutes of Health, Bethesda, USA).

Solubility

Three polytetrafluoroethylene rings 1.5 mm thick with inner diameter of 20 mm were used for each sealer. The rings were placed on a glass plate covered with a cellophane sheet, and were filled with freshly mixed sealers. In sequence a nylon thread was inserted in the softened cement and another cellophane sheet and glass plate were placed onto the samples filled with the sealer. The assembly was placed in an incubator (37°C, 95% relative humidity) for a period corresponding to three times the setting time (72 hours). The sealers were removed from the assembly and weighed three times each with an accuracy of 0.0001 g (UMark 210; Bel Engineering, Monza, Italy). The samples were suspended by the nylon thread and placed 2 × 2 inside a plastic vessel containing 50 mL of deionized distilled water and stored in containers for 24 hours in an incubator (37°C, 95% relative humidity). The samples were rinsed with deionized distilled water, blotted dry with absorbent paper, placed in desiccators for 24 hours, and then reweighed. The experiment was repeated three times for each sealer. The weight loss of each sample (initial mass minus final mass), expressed as the percentage of the original mass was taken as the solubility of the sealer.

Flowability Test

A total of 0.5 mL of sealer was placed on a glass plate (40 × 40 mm^2 top surface) using a graduated disposable 3-mL syringe. Three minutes after the start of mixing, another plate with a mass of 20 ± 2 g and a load of 100 g plus was applied centrally on top of the material. The load was removed 10 minutes after the start of mixing and the average of the major and minor diameters of the compressed discs was measured using a digital calliper with a resolution of 0.01 mm (Mitutoyo MTI Corporation, Tokyo, Japan). If both measurements were consistent to within 1 mm, the results were recorded. If the major and minor diameter discs were not uniformly circular or did not match within 1 mm, the test was repeated. The mean of three measurements for each sealer was taken as the flowability of the material.

Setting Time

Three stainless steel rings with an inner diameter of 10 mm and 2 mm in thickness were used for each

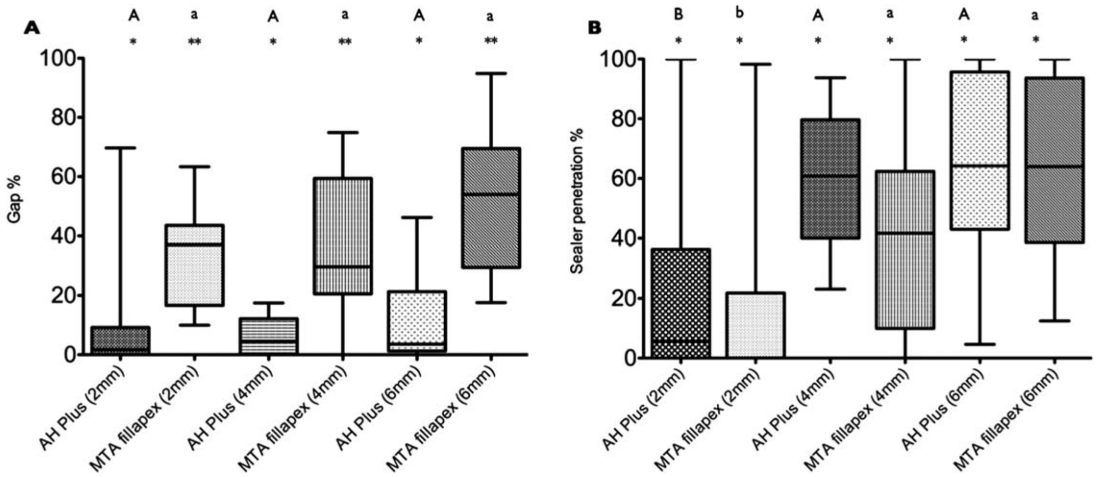

Fig. 1. Box plot illustrating the median, minimum–maximum values and variance of (A) gaps and (B) sealer penetration segments percentiles in the evaluated sections. Different uppercase and lowercase letters in each column indicate statistically significant differences ($P < 0.05$) for the two filling materials (AH Plus–MTA Fillapex) respectively. Also different symbols indicate significant differences between the same group ($P < 0.05$).

sealer. The external borders of the moulds were fixed with wax on a standard optical microscopy glass slide ($25 \times 75 \times 1$ mm^3). The sealers were mixed according to the manufacturer's directions and the moulds were filled. Next, the specimens were stored in an incubator at 37°C and 95% relative humidity. When the setting time stated by the manufacturer approaches, a Gilmore-type needle with a mass of (100 ± 0.5) g having a flat end (2.0 ± 0.1) mm in diameter was carefully lowered vertically onto the horizontal surface of the sealer. The needle tip was cleaned and the movement was repeated until indentations ceased to be visible. The time from the start of mixing to this point was recorded. The arithmetic mean of three repetitions for each sealer was recorded and considered as the final setting time.

Statistical Analysis

For adaptation (gaps) and sealer penetration (SP) between groups, a nonparametric Mann-Whitney test was applied because of the absence of normal distribution confirmed in a preliminary analysis (Shapiro-Wilks test). To compare gaps and SP of the same group sections the Dunn's Multiple comparison test was applied. Physical properties were analyzed by a Student t test. For all tests, the Prisma 5.0 software (GraphPad Software, La Jolla, USA) was used and the statistical significance was established ($P < 0.05$).

RESULTS

Gaps on the Sealer/Dentin Interface and Percentage of Sealer Penetration into Dentinal Tubules

The median and ranges (min–max) from the microscopic analysis are shown in Figure 1. The CLSM analysis of the interfacial region showed higher percentage of gaps at all levels in the MTA Fillapex group. The range of gaps between the filling material and dentinal walls was between 29% and 54% of the root canal perimeter, and when compared to the AH Plus, it was significantly different ($P = 0.0001$). Sealer penetration into the dentinal tubules was statistically similar for both sealers at the 2 mm ($P = 0.16$), 4 mm ($P = 0.15$), and 6 mm ($P = 0.96$) levels, with a lower percentage of penetration at the 2 mm level and the highest at the 6 mm level. The comparison between the sections in the same group showed no statistical differences for gaps ($P < 0.05$), and lower penetration at 2 mm for AH Plus ($P = 0.0006$) and MTA Fillapex ($P = 0.0001$) when compared to the 4 and 6 mm sections (Fig. 2). Representative images of the sealer penetration and interfacial adaptation and are shown in Figures 2 and 3, respectively.

Physical Properties

AH Plus showed a significant low percentage of solubility (0.20 ± 0.01) while MTA Fillapex showed the highest (14.22 ± 1.41) ($P = 0.0001$), which was above the ANSI/ADA requirements. The MTA Fillapex also presented higher values of flowability (41.33 ± 0.76) mm and a lower setting time of (300 ± 3.00) minutes, in comparison to the AH Plus flowability (34.4 ± 4.3) mm and setting time (1337 ± 15.28) minutes. For the setting time, the difference between these sealers was significant ($P = 0.0001$).

DISCUSSION

In our study the null hypothesis was rejected as the MTA Fillapex presented a higher percentage of gaps on the sealer/dentin interface and had significant differences on some physical properties when compared to the AH Plus. This last sealer had been used for comparison with other new sealers because of the low gap percentage and other properties as reported in previous studies (De-Deus et al., 2011; Marciano et al., 2011). During the obturation process the sealer penetrates into the dentinal tubules giving rise to a mechanical interlocking between the sealer and dentin (Haragushiku et al., 2010). However, as the setting process of the resin occurs, the shrinkage-related stress increases gradually and the sealer detaches at the sealer/dentine interface forming gaps (Bergmans et al., 2005). The adhesion

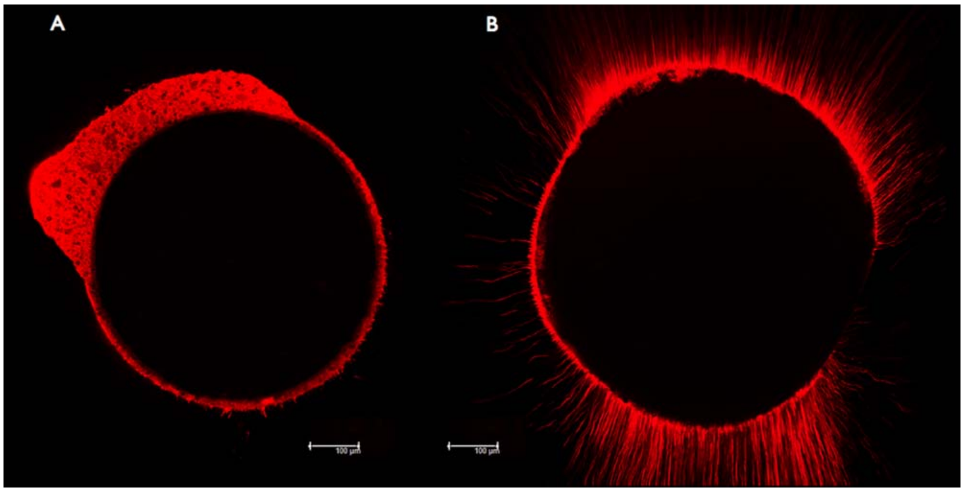

Fig. 2. Representative confocal images of sealer penetration on dentinal tubules of the AH Plus. At 2 mm (A) from the apex, no evident penetration can be observed. Contrary, figure (B) shows a higher penetration of sealer around the root canal section (6 mm). Bars represent 100 μm. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

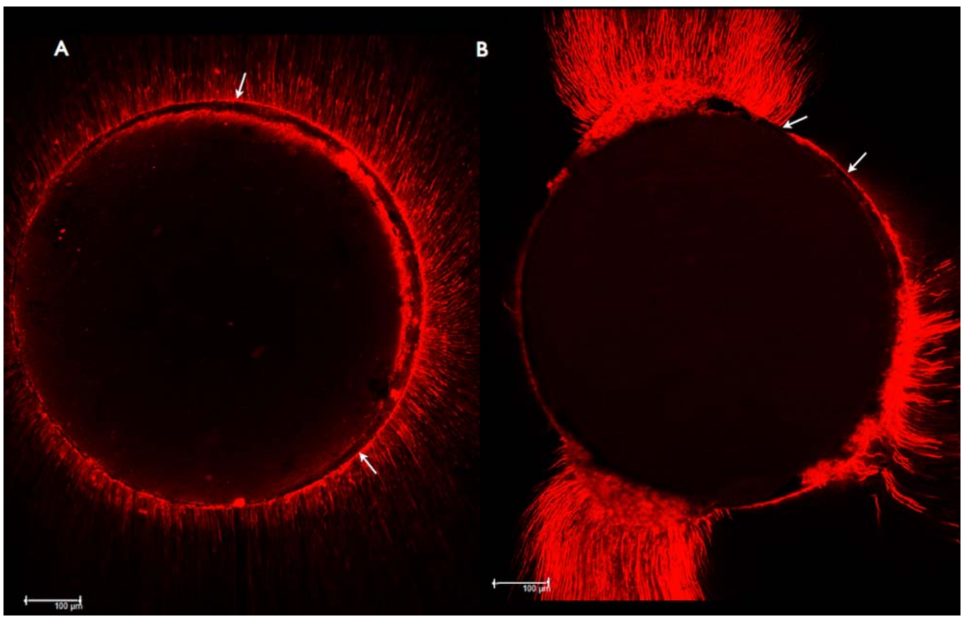

Fig. 3. Representative confocal images of root canal sections (6 mm) filled with the single cone technique using (A) MTA Fillapex and (B) AH Plus. Adaptation failures on the sealer/dentin interface can be observed (arrows). Bars represent 100 μm. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

failures observed in this study revealed a lower percentage of gap containing regions for the AH Plus (< 5%), in accordance to a previous study (De-Deus et al., 2011). On the other hand, a significant higher disadaptation of the MTA Fillapex from the canal walls ranging from 29% to 54% of the canal perimeter is observed. These adaptation failures throughout the obturated canal could increase the potential microbial invasion and recolonization, as bacteria have the ability to enter into the sealer/dentin interface of obturated canals both as biofilm and in planktonic form (Estrela et al., 2009; Roth et al. 2012).

MTA Fillapex contains resinous components (salicylate, diluting and natural resin). Orstavik et al. (2001), reported that sealers containing salicylate in its composition showed initial volumetric shrinkage during the setting reaction, increasing the contraction factor. On the other hand, epoxy resin sealers are considered to have a low contraction factor and some degree of expansion during the setting reaction. Currently, there is scarce literature on the adaptability of the MTA Fillapex to the root canal walls. One study evaluated the adhesion of the MTA Fillapex under various moisture conditions and showed that the dentin surfaces were almost completely devoid of sealer under dry and wet conditions (Nagas et al., 2012).

Nowadays the Atomic force microscope (AFM) is considered a gold standard for the evaluation of dental surfaces and marginal gaps of various dental materials and implants (Cresti Itri et al., 2013; Sharma Cross et al., 2010). However, in our study, CLSM was used because it allows visualization of the sealer penetration in the canal circumference of each section using fluorescence that allowed the formation of high-contrast points to show the sealer distribution within the dentinal tubules. Additionally the samples are not affected by tip shape convolution because of physical probe contact with the sample as it occurs in AFM. Another advantage when using CLSM is that the samples can be visualized in various depths and it can differentiate the genuine interfacial failures avoiding artificial gaps that could be produced after high-vacuum desiccation when scanning electron microscopy is used (Ordinola-Zapata et al., 2009). Moreover, there is no need of special sample processing that can be destructive and produce artifacts. CLSM also permits multiple labeling of different components using different dyes (Kok Rosa et al., 2014).

With regards to the sealer penetration into the dentinal tubules, the CLSM analysis showed similar penetration of both sealers, although it was not continuous throughout the root canal walls. This penetration of the sealer can be influenced by the number of dentinal tubules which decreases from the coronal to the apical portion of the root (Carrigan et al., 1984), as observed in the results of our study, where the major penetration was seen at the 6 mm level and decreased at the 4 and 2 mm levels, respectively (Fig. 2). The sealer penetration into the tubules has the advantage of enhancing the mechanical retention to the dentinal walls (Kokkas et al., 2004; Ordinola-Zapata et al., 2009), and serves as a blocking agent that may prevent bacterial repopulation, or inactivate them inside the tubules. The sealers containing MTA may possess antibacterial activity before setting (Morgental et al., 2011). Additionally, in an *in vitro* study, the MTA Fillapex had a major bacterial biofilm reduction than the AH Plus sealer (Faria-Junior et al., 2013).

It is known that an inflammatory reaction can be initiated by the disaggregation of the material to the periapical region. For that reason the solubility of a sealer must be minimal to avoid leakage that can lead to cytotoxicity. In a previous study, the MTA Fillapex showed severe cytotoxicity when cells were exposed to fresh elute of the sealer and stayed cytotoxic over 4 weeks (Silva et al., 2013). The authors explained that this toxicity might be related to the components of the sealer (salicylate resin, diluting resin, and silica). Viapiana et al. (2013) also explained that the high solubility of the MTA Fillapex may probably be related to the additives that are incorporated in the composition of the sealer that destabilizes its matrix. Furthermore, if the filling is exposed to oral fluids, a high solubility of a sealer may influence the gap formation (Orstavik, 1983).

In our study, the MTA Fillapex presented high solubility after 24 hours (14.22%), more than recommended by the ANSI/ADA specifications. Other studies revealed similar percentages of solubility of the MTA Fillapex, 14.89% (Borges et al., 2012) and 14.94% (Viapiana et al., 2013). In contrast, our results reported significantly less solubility for the AH Plus (0.20%) after 24 hours ($P = 0.0001$), in agreement with a previous study (Versiani et al., 2006). It is worth mentioning that solubility tests are carried out after the setting of the sealers in a controlled ambience. These characteristics do not occur in clinical conditions in where some degree of humidity inside the root canal may exist, and fresh mixed sealer is used during the obturation procedure. Thus, solubility in a clinical situation is probably higher (Bortoluzzi et al., 2009).

According to the ANSI/ADA's specification No. 57 for the flowability test, a disc of at least 20 mm diameter must be obtained. In this study, the MTA Fillapex showed higher flowability values (41.33 mm) than the AH Plus (34.4 mm) but without statistically significant differences ($P = 0.056$) between them. Factors such as the composition, particle size, shear rate, temperature, and time from mixing are mainly associated to the flowability characteristics of endodontic sealers. A satisfactory flowability improves sealer penetration into root canal irregularities, dentinal tubules and increases the mechanical interlocking between the sealer and dentin (Haragushiku et al., 2010) whereas, a higher flow can lead to dimensional changes (contraction) (Benatti et al., 1978). Another disadvantage of using an endodontic sealer with excessive flowability is the risk of apical extrusion, leading to an inflammation reaction of the periapical tissues caused by the cytotoxicity of the sealers.

Finally, the setting time of a sealer is an important factor from the clinical point of view. According to our results, the MTA Fillapex presented 5 hours of setting time. Vitti et al. (2013), found similar setting times of 4.55 hours while other studies reported lower values of 45 minutes (Zhou et al., 2013), and 66 minutes (Viapiana et al., 2013). AH Plus showed similar setting times as previously reported (Baldi et al., 2012). A slow setting time allows for corrections during the obturation, but also if the sealer is not completely set, it may interfere with the restorative procedures, such as the intracanal postpreparation and placement, and may permit dislodgment of the apical gutta-percha. If the canal is also exposed after root canal treatment and the sealer is unset or partially set, coronal or apical penetration of fluids, bacteria or bacterial byproducts through the gaps or voids may occur (Allan et al., 2001).

The MTA Fillapex showed flowability and intratubular penetration similar to the AH Plus. Nevertheless, the MTA Fillapex sealer presented a higher solubility and considerable quantity of gaps between the sealer/dentin interface in relation to the AH Plus sealer. The setting time of the MTA Fillapex was significant lower in comparison to the AH Plus. Clinicians must take in consideration the quality of endodontic sealers since it is essential in the outcome of the root canal filling.

ACKNOWLEDGMENTS

The authors deny any conflicts of interest related to this study.

REFERENCES

ANSI/ADA Specification no. 57, section 5.8. American Dental Association. 2000. Laboratory Testing Methods: Endodontic Filling and Sealing Materials. Endodontic sealing materials.

Allan NA, Walton RC, Schaeffer MA. 2001. Setting times for endodontic sealers under clinical usage and in vitro conditions. J Endod 27: 421–423.

Baldi JV, Bernardes RA, Duarte MA, Ordinola-Zapata R, Cavenago BC, Moraes JC, de Moraes IG. 2012. Variability of physicochemical properties of an epoxy resin sealer taken from different parts of the same tube. Int Endod J 45:915–920.

Benatti O, Stolf WL, Ruhnke LA. 1978. Verification of the consistency, setting time, and dimensional changes of root canal filling materials. Oral Surg Oral Med Oral Pathol 46:107–113.

Bergmans L, Moisiadis P, De Munck J, Van Meerbeek B, Lambrechts P. 2005. Effect of polymerization shrinkage on the sealing capacity of resin fillers for endodontic use. J Adhes Dent 7:321–329.

Borges RP, Sousa-Neto MD, Versiani MA, Rached-Junior FA, De-Deus G, Miranda CE, Pecora JD. 2012. Changes in the surface of four calcium silicate-containing endodontic materials and an epoxy resin-based sealer after a solubility test. Int Endod J 45:419–428.

Bortoluzzi EA, Broon NJ, Bramante CM, Felippe WT, Tanomaru Filho M, Esberard RM. 2009. The influence of calcium chloride on the setting time, solubility, disintegration, and pH of mineral trioxide aggregate and white Portland cement with a radiopacifier. J Endod 35:550–554.

Carrigan PJ, Morse DR, Furst ML, Sinai IH. 1984. A scanning electron microscopic evaluation of human dentinal tubules according to age and location. J Endod 10:359–363.

Cresti S, Itri A, Rebaudi A, Diaspro A, Salerno. M 2013. Microstructure of Titanium-Cement-Lithium Disilicate Interface in CAD-CAM Dental Implant Crowns: A Three-Dimensional Profilometric Analysis. Clin Implant Dent Relat Res 11:2523–2545.

D'Alpino PH, Pereira JC, Svizero NR, Rueggeberg FA, Pashley DH. 2006. Use of fluorescent compounds in assessing bonded resin-based restorations: A literature review. J Dent 34:623–634.

De-Deus G, Reis C, Di Giorgi K, Brandao MC, Audi C, Fidel RA. 2011. Interfacial adaptation of the Epiphany self-adhesive sealer to root dentin. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 111:381–386.

Ersahan S, Aydin C. 2013. Solubility and apical sealing characteristics of a new calcium silicate-based root canal sealer in comparison to calcium hydroxide-, methacrylate resin- and epoxy resin-based sealers. Acta Odontol Scand 71:857–862.

Estrela C, Sydney GB, Figueiredo JA, Estrela CR. 2009. A model system to study antimicrobial strategies in endodontic biofilms. J Appl Oral Sci 17:87–91.

Faria-Junior NB, Tanomaru-Filho M, Berbert FL, Guerreiro-Tanomaru JM. 2013. Antibiofilm activity, pH and solubility of endodontic sealers. Int Endod J 46:755–762.

Gatewood RS. 2007. Endodontic materials. Dent Clin North Am 51: 695–712.

Grossman LI, Oliet S, Del Rio CE. 1988. Endodontic practice, 11th ed. Philadelphia: Lea and Febiger.

Haragushiku GA, Sousa-Neto MD, Silva-Sousa YT, Alfredo E, Silva SC, Silva RG. 2010. Adhesion of endodontic sealers to human root dentine submitted to different surface treatments. Photomed Laser Surg 28:405–410.

Hargreaves KM, Cohen S, Berman LH. 2011. Cohen's pathways of the pulp, 10th ed. St. Louis, Mo: Mosby Elsevier.

Kok D, Rosa RA, Barreto MS, Busanello FH, Santini MF, Pereira JR, Só MVR. 2014. Penetrability of AH plus and MTA fillapex after endodontic treatment and retreatment: A confocal laser scanning microscopy study. Microsc Res Tech 77:467–471.

Kokkas AB, Boutsioukis A, Vassiliadis LP, Stavrianos CK. 2004. The influence of the smear layer on dentinal tubule penetration depth by three different root canal sealers: an in vitro study. J Endod 30: 100–102.

Marciano MA, Guimaraes BM, Ordinola-Zapata R, Bramante CM, Cavenago BC, Garcia RB, Bernardineli N, Andrade FB, Moraes IG, Duarte MA. 2011. Physical properties and interfacial adaptation of three epoxy resin-based sealers. J Endod 37:1417–1421.

Morgental RD, Vier-Pelisser FV, Oliveira SD, Antunes FC, Cogo DM, Kopper PM. 2011. Antibacterial activity of two MTA-based root canal sealers. Int Endod J 44:1128–1133.

Nagas E, Uyanik MO, Eymirli A, Cehreli ZC, Vallittu PK, Lassila LV, Durmaz V. 2012. Dentin moisture conditions affect the adhesion of root canal sealers. J Endod 38:240–244.

Ordinola-Zapata R, Bramante CM, Graeff MS, del Carpio Perochena A, Vivan RR, Camargo EJ, Garcia RB, Bernardineli N, Gutmann JL, de Moraes IG. 2009. Depth and percentage of penetration of endodontic sealers into dentinal tubules after root canal obturation using a lateral compaction technique: a confocal laser scanning microscopy study. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 108:450–457.

Orstavik D. 1983. Weight loss of endodontic sealers, cements and pastes in water. Scand J Dent Res 91:316–319.

Orstavik D, Nordahl I, Tibballs JE. 2001. Dimensional change following setting of root canal sealer materials. Dent Mater 17: 512–519.

Ricucci D, Lin LM, Spangberg LS. 2009. Wound healing of apical tissues after root canal therapy: A long-term clinical, radiographic, and histopathologic observation study. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 108:609–621.

Roth KA, Friedman S, Levesque CM, Basrani BR, Finer Y. 2012. Microbial biofilm proliferation within sealer-root dentin interfaces is affected by sealer type and aging period. J Endod 38: 1253–1256.

Sharma S, Cross SE, Hsueh C, Wali RP, Stieg AZ, Gimzewski JK. 2010. Nanocharacterization in dentistry. Int J Mol Sci 11:2523–2545.

Silva EJ, Rosa TP, Herrera DR, Jacinto RC, Gomes BP, Zaia AA. 2013. Evaluation of Cytotoxicity and Physicochemical Properties of Calcium Silicate-based Endodontic Sealer MTA Fillapex. J Endod 39:274–277.

Versiani MA, Carvalho-Junior JR, Padilha MI, Lacey S, Pascon EA, Sousa-Neto MD. 2006. A comparative study of physicochemical properties of AH Plus and Epiphany root canal sealants. Int Endod J 39:464–471.

Viapiana R, Flumignan DL, Guerreiro-Tanomaru JM, Camilleri J, Tanomaru-Filho M. 2013. Physicochemical and mechanical properties of zirconium oxide and niobium oxide modified Portland cement-based experimental endodontic sealers. Int Endod J 47: 437–448.

Vitti RP, Prati C, Sinhoreti MA, Zanchi CH, Souza ESMG, Ogliari FA, Piva E, Gandolfi MG. 2013. Chemical-physical properties of experimental root canal sealers based on butyl ethylene glycol disalicylate and MTA. Dent Mater 29:1287–1294.

Zhou HM, Shen Y, Zheng W, Li L, Zheng YF, Haapasalo M. 2013. Physical properties of 5 root canal sealers. J Endod 39:1281–1286.

Zmener O, Martinez Lalis R, Pameijer CH, Chaves C, Kokubu G, Grana D. 2012. Reaction of rat subcutaneous connective tissue to a mineral trioxide aggregate-based and a zinc oxide and eugenol sealer. J Endod 38:1233–1238.