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RESEARCH ARTICLE

Penetrability of a new endodontic sealer: A confocal laser scanning microscopy evaluation

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Abstract

This study aimed to evaluate the penetration of a new endodontic sealer into the dentinal tubules. Twenty single-rooted teeth were selected. The crown was sectioned, and the canals were instrumented with a reciprocating system. Irrigation was performed with 2 mL of 2.5% sodium hypochlorite between each file change. After instrumentation, the root canals were irrigated with 2 mL of17% EDTA for 3 min followed by saline solution. The specimens were randomized into two groups (n =10) according to the endodontic sealer: AH Plus (AP) or Sealer Plus (SP). All specimens were filled using the lateral compaction technique. Rhodamine B dye (red) was incorporated to the sealers to provide the fluorescence which will enable confocal laser scanning microscopy (CLSM) assessment. The roots were sectioned 2, 4, and 6 mm from the apex and assessed by CLSM. The root canal level affected the penetration of the sealer, but no statistical significant differences were found between the two experimental groups (p > .05). SP presented similar dentinal penetration and perimeter integrity to the gold standard (AP).

Research Highlights

Sealer Plus presents dentinal penetrability and perimeter integrity similar to the gold standard sealer (AH Plus), demonstrating excellent ability of filling areas of difficult access.

KEYWORDS

confocal microscopy, endodontics, root canal filling materials

1 | INTRODUCTION

The success of the endodontic therapy is based on different steps that must be executed correctly: instrumentation (Yee, Newton, Patterson, & Swartz, 1984), irrigation (Goldberg, Bernat, Spielberg, Massone, & Piovano, 1985), filling technique (Russin, Zardiackas, Reader, & Menke, 1980) and as part of it, the type of the sealer (Alexander & Gordon, 1985), which may influence the sealability of the root canal filling.

Ideally, proper sealing is that in which the endodontic sealer could penetrate into infected dentine tubules, acting as a physical barrier. Then, it would provide an apical seal and prevent remaining bacteria and their toxins invading the apex what could contribute to the endodontic therapy failure (Ordinola-Zapata et al., 2009; Pommel, Jacquot, & Camps, 2001). The sealer penetration into dentinal tubules is influenced by different factors as smear layer removal (White, Goldman, & Lin, 1984; White, Goldman, & Lin, 1987), dentine permeability (Oksan, Aktener, Sen, & Tezel, 1993), filling technique (De Deus, Gurgel-Filho, Maniglia-Ferreira, & Coutinho-Filho, 2004), and physic-chemical properties (Oksan et al., 1993).

The new endodontic sealer, Sealer Plus, besides being the base of epoxy resin has calcium hydroxide in its composition which makes it less cytotoxic, that is, more biocompatible (Cintra et al., 2017). In addition, the sealer exhibits physicochemical properties according to No. 57 of ANSI/ADA and ISO6876 and also good solubility, radiopacity, and pH (Vertuan et al., 2017).

Epoxy resin-based sealers present great apical sealing compared to others endodontic sealers (De Almeida, Leonardo, Tanomaru Filho, & Silva, 2000), although other sealers also must promote an utterly homogeneous interface between the sealer and root dentin, with a consistent adaptation of sealer tags (Ordinola-Zapata et al., 2009). The aim of this study was to evaluate the penetrability of a new epoxy-based sealer (Sealer Plus - MK Life, Porto Alegre, RS, Brasil) into dentinal tubules compared to the gold standard sealer (AH Plus - Dentsply Maillefer, Ballaigues, Suiça).

2 | MATERIAL AND METHODS

This study was submitted and approved by Ethics Committee of Bauru Dental College (080/2011). Twenty single-rooted teeth were selected. Teeth were disinfected in sodium hypochlorite for 24 hr and stored in 0.2% sodium for 2 days before canal preparation, and then stored in saline solution. Only extracted teeth with straight roots, without external or internal root resorption, and absence of anatomic variations were included.

The teeth were prepared sectioning the crowns and then the canals were explored using a #15K-file (Dentsply Maillerfer, Ballaigues, Switzerland) until its tip became visible at the foraminal opening. Next, the working length was established 1 mm shorter and the apical size was standardized in a diameter 40 because of the file used during canal preparation. The canal preparation was performed with the reciprocating Unicone rotary files 40.06 (MEDIN, Nové Město na Moravě, Czech Republic) activated by an electric motor (VDW Silver; VDW Company, Munich, Germany) using the "WaveOne all" mode. During preparation, each sample was irrigated with 5 mL of 2.5% sodium hypochlorite. After instrumentation, the root canals were irrigated with 2 mL of 17% EDTA for 3 min, and the final irrigation was performed by saline solution.

The specimens were randomly divided into two groups (n = 10), according to the endodontic sealer: AH Plus (AP) and Sealer Plus (SP). Root canal filling was performed by a single operator, using the lateral compaction technique. The master cone was coated with the sealer and placed into the root canal; then cold lateral compaction was performed using a finger spreader size B (Dentsply Maillefer). Rhodamine B at a proportion of 0.01% was incorporated to the sealer for confocal laser scanning microscopy (CLSM) improvement.

Specimens were stored at 100% humidity and 36°C. After 7 days, the roots were transversally cleaved at a cut machine (Isomet, Buehler, Ill) under water cooling at 2, 4, and 6 mm from the apex. Slices were assessed from coronal to the apex surfaces with a scanning confocal microscope (Leica Microsystens, Mannheim, Germany). The emission wavelengths were 545/740 nm. Dentin samples were analyzed using the ×10 lens.

Sealer penetration and empty areas were analyzed by ImageJ V1.46r software (US National Switzerland). For examiner's calibration, was used the tool "spatial calibration" and a line was drawn on an established scale in the CLSM software image, equivalent to 200 μm. The tool "distance" was used to measure the longest sealer penetration point between canal wall and the external root surface at four dimensions (buccal, lingual, mesial, distal). Freehand line selection tool outlined the root canal and endodontic sealer perimeter, and so, the integrity of the sealer perimeter was calculated. The Kolgomorov-Smirnov normality was used to verify the distribution of the data. Then, Student t-test was used to compare the sealers in each root level and one-way ANOVA and Tukey post hoc tests were used to compare the root levels within the same sealer. Percentage of perimeter integrity was assessed with Mann–Whitney U test. The significance level was set at 5%.

3 | RESULTS

When the sealers were compared within the same root level, no differences were observed regarding the mean of sealer penetration and integrity of the sealer perimeter (p > .05) (Table 1). The root level affected the sealer penetration for both sealers. The mean of dentinal penetration in the apical third was lower than that observed in cervical third (P 0.05). Figure 1 shows representative CLSM images of SP and AP groups in the three root levels. SP and AP presented greater penetration in the vestibular and lingual aspects of the roots, and good filling ability in isthmus areas.

4 | DISCUSSION

Anatomic complexities, such as dentinal tubules, isthmuses, lateral canals, and apical ramifications protected bacterias from chemomechanical procedures (Vera et al., 2012). Endodontic sealers assume importance for "entombing" residual microorganisms in those areas inaccessible by the instruments and gutta-percha filling (Kara Tuncer, Tuncer, & Gökyay, 2014; Vertuan et al., 2017). The sealer penetration depends on many factors, including the effectiveness of the removal of the smear layer, the physical and chemical properties of the sealer, and the anatomy of the root canal system. (Kara Tuncer & Tuncer, 2012).

In this study, the final irrigation was performed with 17% EDTA solution with the aim to improve dentinal permeability, and high

TABLE 1 Mean values and standard deviations (SD) from the depth sealer penetration (μm) into dentinal tubules and median of the percentage of integrity of sealer perimeter according to the groups and root third (N = 20)

	Cervical		Medium		Apical	
	Sealer Penetration	Sealer Perimeter	Sealer Area	Sealer Perimeter	Sealer Penetration	Sealer Perimeter
AH plus	989.4[a] (345.7)	96.2% (±7.2)	678.0 (353.75)	99.1% (±2.6)	296.5[a] (±393.5)	85.9% (±16.9)
Sealer plus	849.2[b] (356.6)	96.1% (±6.8)	491.3 (406.1)	93.2% (±12.8)	268.18[b] (±302.0)	86.9% (±30.0)

Note. Different letters in the column denote the presence of differences between the groups (p < .05).

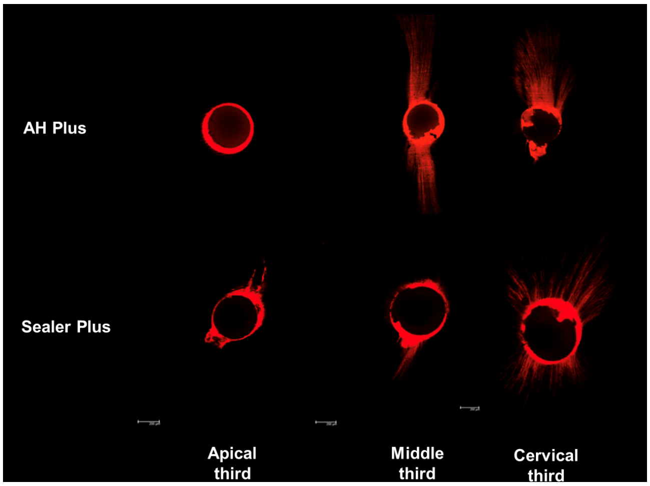

FIGURE 1 CLSM representative images of the sealer penetration and perimeter integrity of SP and AP groups in the three root levels [Color figure can be viewed at wileyonlinelibrary.com]

means of sealer penetration were observed for both groups. The results are in agreement with others authors who demonstrated that regardless of the root canal sealer, smear layer removal is mandatory to improve sealer penetration into dentinal tubules (Ordinola-Zapata et al., 2009; Sabadin, Böttcher, Hoppe, Santos, & Grecca, 2014).

Although no difference in dentinal penetration was found among SP and AP, the results indicate differences between thirds analyzed. Anatomical variations explained low penetration on apical third when compared to the cervical level. The number of the tubules and its size are reduced in this area, and the difficulty in delivering the irrigant in the apical third reduce the effectiveness of chemical solutions and instruments, which could affect smear layer removal in this area (Balguerie, van der Sluis, Vallaeys, Gurgel-Georgelin, & Diemer, 2011; De Deus et al., 2004; Kara Tuncer & Tuncer, 2012; Sonu, Girish, Ponnappa, Kishan, & Thameem, 2016). High penetration observed on cervical third could be explained mainly by the chelating action (De Deus et al., 2004).

Another essential characteristic of the sealers which contribute to their dentinal penetration is the flow rate. It consists of a physical property that indicates the capacity of the sealer penetrates in areas of complex anatomy (Grossman, 1976; Solomonov & Itzhak, 2017), an important factor in the filling of lateral or accessory canals. The flow property of endodontic sealers depends on the composition, setting time, and it can be improved by pressure (Grossman, 1976). AH Plus is considered the gold standard sealer for comparison to others brands or chemical composition. AH Plus has an optimal flow rate and tubular penetration (Sonu et al., 2016), this could be associated with the structural integrity of the epoxy resin-based sealer that provides depth penetration of the sealer (White et al., 1987).

According to the results of the present study, both groups present great penetration in isthmus areas and both sealers covered almost completely the gutta-percha cones, and filled empty spaces between gutta-percha and dentin. These results were similar to the findings of Kok et al. (2012). These authors observed that the cold lateral compaction using AH Plus sealer presents integrity of the sealer perimeter at apical third close to 100%. Both groups showed unequal sealer distribution around the root. Greater dentinal penetration was observed in the vestibular and lingual aspect of the roots. In concordance with our findings, Generali, Prati, Cavani, Gatto, and Gandolfi (2017) found this unexpected distribution and associated this phenomenon to a "butterfly effect" (Russel, Chandler, Haumann, Siddiqui, & Tompkins, 2013), which is due to the differences of the density of dentinal tubules and sclerotic dentine, that may affect the sealer penetration.

Sealer Plus is a new endodontic epoxy resin-based sealer recently introduced into the market. There is only one study on the literature which evaluated some of the physicochemical properties of this new sealer in comparison with AH Plus. Vertuan et al. (2017) found physicochemical properties in accordance with the specification of American Dental Association (2000) and ISSO 6876 for Sealer Plus (International Organization for Standardization, 2012). Among properties analyzed, a higher flow value was observed for AH Plus sealer when compared to Sealer Plus. In the present study, despite the different flow rate, SP and AP showed similar penetration into dentinal tubules, demonstrating that the new sealer has excellent ability of filling areas of difficult access.

To develop a new endodontic sealer, it's necessary to evaluate its physicochemical properties which are fundamental to the sealer clinical behavior (Solomonov & Itzhak, 2017). Laboratory studies could contribute to a better understanding of the clinical behavior prior the market launch by the manufactures and the clinicians. CLSM allows visualization of the depth penetration and adaptation of sealer adaptation into the root canal and dentinal tubules (Ordinola-Zapata et al., 2009). As an advantage, this methodology does not require sample preparation, is a nondestructive process and keeps the sealers integrity thus allowing accurate measurement of sealer penetration depths (Kara Tuncer & Tuncer, 2012; Sonu et al., 2016).

5 | CONCLUSION

The knowledge of the properties of the sealers by dentists improves the clinical success; as well provide conditions to manufacturer development of better materials. Based on the results of this in vitro study, SP presented dentinal penetrability and perimeter integrity similar to those achieved with AP. The root canal level affected the penetration of the sealer.

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REFERENCES

Alexander, J. B., & Gordon, T. M. (1985). A comparison of the apical seal produced by two calcium hydroxide sealers and a Grossman-type sealer when used with laterally condensed gutta-percha. *Quintessence International*, *16*, 615–621.

American National Standards/American Dental Association (2000). Specification no. In *57 for endodontic sealing materials*. Chicago, IL: American National Standards/American Dental Association.

Balguerie, E., van der Sluis, L., Vallaeys, K., Gurgel-Georgelin, M., & Diemer, F. (2011). Sealer penetration and adaptation in the dentinal tubules: A scanning eletron microscopic study. *Journal of Endodontia*, *37*, 1576–1579.

Cintra, L. T. A., Benetti, F., de Azevedo Queiroz, Í. O., Ferreira, L. L., Massunari, L., Bueno, C. R. E., … Gomes-Filho, J. E. (2017). Evaluation of the cytotoxicity and biocompatibility of new resin epoxy-based endodontic sealer containing calcium hydroxide. *Journal of Endodontics*, *43*, 2088–2092. https://doi.org/10.1016/j.joen.2017.07.016

De Almeida, W. A., Leonardo, M. R., Tanomaru Filho, M., & Silva, L. A. B. (2000). Evaluation of apical sealing of three endodontic sealers. *International Endodontic Journal*, *33*, 25–27.

De Deus, G. A., Gurgel-Filho, E. D., Maniglia-Ferreira, C., & Coutinho-Filho, T. (2004). The influence of filling technique on depth of tubule penetration by root canal sealer: A study using light microscopy and digital image processing. *Australian Endodontic Journal*, *30*, 23–28.

Generali, L., Prati, C., Cavani, F., Gatto, M. R., & Gandolfi, M. G. (2017). Double dye technique and fluid filtration test to evaluation sealing ability of an endodontic sealer. *Clinical Oral Investigations*, *21*, 1267–1276. https://doi.org/10.1007/s00784-016-1878-0

Goldberg, F., Bernat, M. I., Spielberg, C., Massone, E. J., & Piovano, S. A. (1985). Analysis of the effect of ethylenediaminetetraacetic acid on the apical seal of root canal fillings. *Journal of Endodontia*, *11*, 544–547.

Grossman, L. I. (1976). Physical properties of root canal cements. *Journal of Endodontia*, *2*, 166–175.

International Organization for Standardization. (2012). *ISO 6876: Dental root canal sealing materials*. Geneva, Switzerland: International Organization for Standardization.

Kara Tuncer, A., & Tuncer, S. (2012). Effect of different final irrigation solutions on dentinal tubule penetration depth and percentage of root canal sealer. *Journal of Endodontia*, *38*, 860–863.

Kara Tuncer, A., Tuncer, S., & …Gökyay, S. S. (2014). Correlation between sealer penetration into dentinal tubules and bond strength of two new calcium silicate-based and an epoxy resin-based, endodontic sealer. *Journal of Adhesion Science and Technology*, *28*, 702–710.

Kok, D., Húngaro Duarte, M. A., Abreu Da Rosa, R., Wagner, M. H., Pereira, J. R., & Só, M. V. (2012). Evaluation of epoxy resin sealer after three root canal filling techniques by confocal laser scanning microscopy. *Microscopy Research and Technique*, *75*, 1277–1280.

Oksan, T., Aktener, B. O., Sen, B. H., & Tezel, H. (1993). The penetration of root canal sealers into dentinal tubules. A scanning electron microscopic study. *International Endodontic Journal*, *26*, 301–305.

Ordinola-Zapata, R., Bramante, C. M., Graeff, M. S., del Carpio Perochena, A., Vivan, R. R., Camargo, E. J., … de Moraes, I. G. (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 Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontics*, *108*, 450–457.

Pommel, L., Jacquot, B., & Camps, J. (2001). Lack of correlation among three methods for evaluation of apical leakage. *Journal of Endodontia*, *27*, 347–350.

Russin, T. P., Zardiackas, L. D., Reader, A., & Menke, R. A. (1980). Apical seals obtained with laterally condensed, chloroform-softened gutta-percha and laterally condensed gutta-percha and Grossman's sealer. *Journal of Endodontia*, *6*, 678–682.

Sabadin, N., Böttcher, D. E., Hoppe, C. B., Santos, R. B., & Grecca, F. S. (2014). Resin-based sealer penetration into dentinal tubules after the use of 2% chlorhexidine gel and 17% EDTA: in vitro study. *Brazilian Journal of Oral Sciences*, *13*, 308–313.

Solomonov, M., & Itzhak, J. B. (2017). Evaluating the physical properties of one novel and two well-established epoxy resin-based root canal sealers. *ENDO*, *11*, 285–290.

Sonu, K. R., Girish, T. N., Ponnappa, K. C., Kishan, K. V., & Thameem, P. K. (2016). Comparative evaluation of dentinal penetration of three different endodontic sealers with and without smear layer removal – Scanning electron microscopic study. *Saudi Endodontic Journal*, *6*, 16–20. https://doi.org/10.4103/1658-5984.171996

Vera, J., Siqueira, J. F., Ricucci, D., Loghin, S., Fernández, N., Flores, B., & Cruz, A. G. (2012). One-versus two-visit endodontic treatment of teeth with apical periodontitis: A histobacteriologic study. *Journal of Endodontia*, *38*, 1040–1052. https://doi.org/10.1016/j.joen.2012.04.010

Vertuan, G. C., Duarte, M. A. H., Moraes, I. G., Piazza, B., Vasconcelos, B. C., Alcade, M. P., & Vivan, R. R. (2017). Evaluation of physicochemical properties of a new root canal sealer. *Journal of Endodontia*, *44*, 501–505. https://doi.org/10.1016/j.joen.2012.04.010

Russel, A. A., Chandler, N. P., Haumann, C., Siddiqui, A. Y., & Tompkins, G. R. (2013). The butterfly effect: An investigation of sectioned roots. *Journal of Endodontia*, *39*, 208–210. https://doi.org/10.1016/j.joen.2012.09.016

White, R. R., Goldman, M., & Lin, P. S. (1984). The influence of the smeared layer upon dentinal tubule penetration by plastic filling materials. *Journal of Endodontia*, *10*, 558–562.

White, R. R., Goldman, M., & Lin, P. S. (1987). The influence of the smeared layer upon dentinal tubule penetration by endodontic filling materials. Part II. *Journal of Endodontia*, *13*, 369–374.

Yee, R. D., Newton, C. W., Patterson, S. S., & Swartz, M. L. (1984). The effect of canal preparation on the formation and leakage characteristics of the apical dentin plug. *Journal of Endodontia*, *10*, 308–317.

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