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SEM Evaluation of the Interface Between Filling and Root-End Filling Materials

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Summary: The aim of this *ex vivo* study was to evaluate, by scanning electron microscopy (SEM), the presence of gaps at the interface between filling material and three root-end filling materials. Thirty human upper molars disto-buccal roots were instrumented and filled with gutta-percha and eugenol-based sealer. The apicoectomy was performed 2 mm from the apex and retrograde cavities were prepared with ultrasonic points (3 mm in deep). The samples were divided into three experimental groups ($n = 10$): Group I—white mineral trioxide aggregate (MTA); Group II—Super EBA; and Group III—Portland cement. The root-end filling materials were inserted into the retocavities using a MTA carrier. After 48 h, the roots were transversally sectioned in order to obtain the apical 5 mm. Next, each specimen was prepared longitudinally with crescent granulation of abrasives water-wet sandpapers in order to expose the filling and root-end filling materials. Then, the specimens were subjected to slow dehydration with silica gel, mounted onto specific stubs and coated with paladium coverage for SEM analysis of the interface between filling and root-end filling materials. The percentage of gaps at the interfacial area was calculated by using Image Tool 3.0 software. Super EBA presented the higher percentage of gaps ($1.5 \pm 0.67\%$), whereas MTA presented the lowest values ($0.33 \pm 0.20\%$; $p = 0.0004$). Despite the statistical differences observed between Super EBA and MTA, all the root-end filling materials presented great adaptation to the filling material, presenting small amount of gaps. SCANNING 36: 252–257, 2014. © 2013 Wiley Periodicals, Inc.

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Introduction

Although endodontic treatment present high success rates, when the biological and technical aspects are not respect, the root canal system contamination remains and may leads to failure (Lazarski *et al*., 2001). In these cases, the nonsurgical root canal retreatment is indicated to re-establish the health of the periapical tissues. However, when the goals of nonsurgical retreatment are not reached or even when this procedure is contra-indicated, periapical surgery may be the only treatment option (Nair *et al*., 2011).

The aim of periapical surgery is to remove diseased tissues and obtain an apical seal to prevent the leakage of microorganisms and residual irritants into the periradicular area (Kim and Kratchman, 2006). For this reason, normally the apical 3 mm of the root is resected, a retrograde preparation is performed, and a root-end filling material is placed into the cavity to promote adequate apical sealing (Nair *et al*., 2011). Therefore, the root-end filling material must present some properties such as biocompatibility to allow or induce bone repair, radiopacity, easy handling, insolubility, dimensional stability, adequate sealing, and great adaptation to the root canal walls and to the filling material (Johnson, ’99).

Several materials have been used as root-end filling materials, including amalgam, zinc oxide and eugenol-based cements, mineral trioxide aggregate (MTA), and Portland cement. Super-EBA was developed from the enhancement of some of zinc oxide and eugenol-based cement properties. Its liquid contains 32% eugenol and 68% ethoxybenzoic acid (EBA) (Oynick and Oynick, ’78). It presents good apical sealing and marginal adaptation, biocompatibility and low cytotoxicity (Olsen *et al*., ’94; Balto and Al-Nazhan, 2003).

MTA is composed of a powder (black or gray) that solidifies into hard structure when in contact with water. It is composed of tricalcium silicate, tricalcium aluminate, tricalcium oxide and silicate oxide, and bismuth oxide used to provide radiopacity to the material. Besides, it provides satisfactory sealing ability (Torabinejad *et al.*, '95; Al-Hezaimi *et al.*, 2005; Chittoni *et al.*, 2012) and adequate biocompatibility (Bernabé *et al.*, 2005). Because MTA is composed mainly by Portland cement, several studies have compared the physical and chemical properties of both cements (Estrela *et al.*, 2000; Dammaschke *et al.*, 2005). Moreover, Portland cement has not a direct dental use, it is essential to test this material with *in vitro* and *in vivo* experiments before using in clinical practice.

Several studies have investigated the adaptation of root-end filling materials with the root dentin (Badr, 2010; Reyes-Carmona *et al.*, 2010); however, up to date, no study has evaluated the adaptation of root-end filling materials to the filling material. Therefore, the aim of this study was to assess, by scanning electron microscopy (SEM), the presence of gaps at the interface between filling material and three root-end filling materials. The hypothesis of this study was that there are statistically significant differences among the three root-end filling materials.

Materials and Methods

Sample Selection, Root Canal Preparation, and Filling

This study was submitted to and approved by the Ethics Committee in Research of the Federal University of Rio Grande do Sul. Thirty disto-bucal roots from human upper molars were stored in saline solution and then, in 2% hypochlorite sodium (NaOCl) for 48 h. The specimens were transversely sectioned at the cement–enamel junction with a diamond disc under water cooling. The working length (WL) was established by inserting a #15 K-file (Dentsply-Maillefer, Ballaigues, Switzerland) until it was visible at the apical foramen. Then, 1 mm was subtracted from this measure to obtain the WL. The root canal preparation was performed up to a size #40 K-file. After each instrument change, irrigation with 2 ml of 1% NaOCl was performed using disposable 5 ml syringes (Ultradent Products, South Jordan, UT) and 30-gauge needles (Endo Eze Tip, Ultradent Products). Thereafter, the canals were dried with paper points (Tanariman, Manacapuru, AM, Brazil) and filled with eugenol-based sealer (Endofill, Dentsply-Maillefer), a size #40 main gutta-percha cone (Tanariman) and B7 accessories gutta-percha cones (Tanariman) using lateral compaction technique. The specimens were stored at 37°C for 24 h to allow the sealer to set.

Apicoectomy, Retrocavities Preparation, and Root-End Fillings

The apicoectomy was performed using Endo Z bur (Dentsply-Maillefer) perpendicular to the long axis of the root and 2 mm short of the apex. Retrograde cavities with 3 mm in deep were subsequently prepared using ultrasound (CVDent 1000; CVDentus, São José dos Campos, SP, Brazil) and ultrasound tips (TOP-2, CVDentus) with under saline cooling. Then, the roots were randomly divided into three experimental groups (n = 10), according with the root-end filling materials: Portland cement (Votorantin, São Paulo, SP, Brazil); MTA (Angelus, Londrina, PR, Brazil) and Super EBA (Harry J. Bosworth Co., Skokie, IL). Portland cement and MTA were manipulated according to the manufacturers' instructions. Super EBA was manipulated to a powder/liquid proportion of 1 g per 0.2 ml (Bernabé *et al.*, 2005). Next, the cement was inserted into root-end cavity using a MTA carrier (Angelus) and a spatula was used to compact the materials vertically. The specimens were stored for 1 week at 37°C in plastic containers with gauzes soaked in distilled water.

Scanning Electron Microscopy (SEM)

The roots were transversally sectioned with a diamond disc (KG Sorensen, Cotia, SP, Brazil) in order to obtain blocks with 5 mm in length containing the apical third (filling and root-end filling materials). Next, the blocks were longitudinally sectioned so that the section did not reach the root canal. Then, each specimen was sanded using water-wet sandpaper (3 M, St. Paul, MN) with crescent grit numbers (800, 1,200, 1,500, 2,000, and 2,500) in a polishing machine (Buehler, Lake Bluff, IL) to exposure the area of interest. A final polishing was performed with 1/4 μm granulation paste (Arotec, Cotia, SP, Brazil; Fig. 1).

The specimens underwent to slow dehydration with silica gel, mounted onto specific stubs for observation under the JEOL 6060 scanning electronic microscope (SEM; JEOL, Tokyo, Japan) and coated only with paladium coverage (MED 010; Balzers Union, Balzers, Liechtenstein, Germany).

SEM Analysis and Statistics

Initially, the electron micrographs were obtained with 30× magnification. Next, the presence of gaps at the interface between filling and root-end filling materials was assessed by quantitative measures with 100× magnification. The image analysis was performed according to Chittoni *et al.* (2012). One blinded and trained examiner analyzed the electron micrographs using the ImageTool 3.0 software (USA). Three readings were performed at an interval of 48 h between each one.

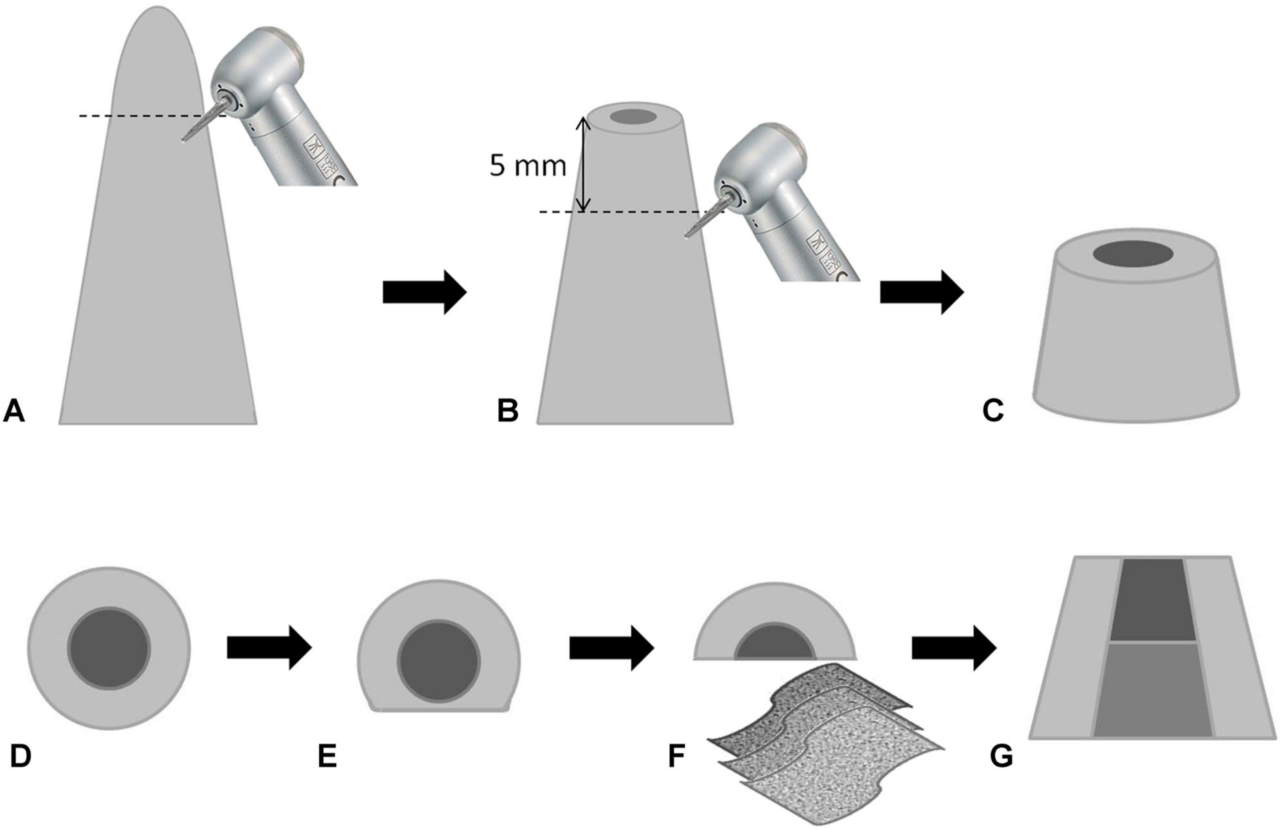

Fig 1. Schematic representation of the steps prior to SEM analysis. (A) Apicoectomy 2 mm short of the apex; (B) transverse sectioning to obtain blocks with 5 mm in length containing the apical third (filling and root-end filling materials); (C) 5 mm blocks; (D) apical view of the specimen; (E) longitudinal section of the specimens so that the section did not reach the root canal; (F) specimens were sanded using water-wet sandpaper with crescent grit numbers to exposure the area of interest; and (G) lateral view of the specimens (interface between filling and root-end filling materials).

Initially, the software's measurement tools were calibrated to present the measures in mm^2. Thereafter, the overall area of the apical third visualized at the images was delimited. This measure corresponds to the 100%. Next step was to measure the empty spaces between the filling and root-end filling materials (i.e., gaps). Finally, the percentage of the gaps was obtained from the ratio between the gap areas (mm^2) and the overall area of the apical third (mm^2).

Statistical analysis was performed by SPSS software package, Version 17.0 for Windows (SPSS Inc., Chicago, IL). The data were analyzed using the Kruskal–Wallis test followed by the post hoc Dunn test for significant differences between groups. Level of significance was set at 5%.

Results

The highest gap areas were recorded for Super EBA and the lowest for MTA, with statistically significant differences ($p = 0.0004$). Although MTA had presented lower gap areas than Portland cement, there were no significant differences between them ($p > 0.05$). The means and standard deviations of the gap areas were summarized in Table I. Figure 2 represent SEM imaging

TABLE I Median and standard deviation of gap areas (in percentage) between the filling and root-end fillings materials

	Mean ± SD	Median	25th percentile	75th percentile
Super EBA	1.58 ± 0.67^{a}	1.90	0.94	2.01
MTA	0.33 ± 0.20^{b}	0.28	0.24	0.31
Portland	0.58 ± 0.13^{ab}	0.61	0.48	0.70

Different superscript letters indicate statistically significant difference at 5% significance level (Kruskal–Wallis test).

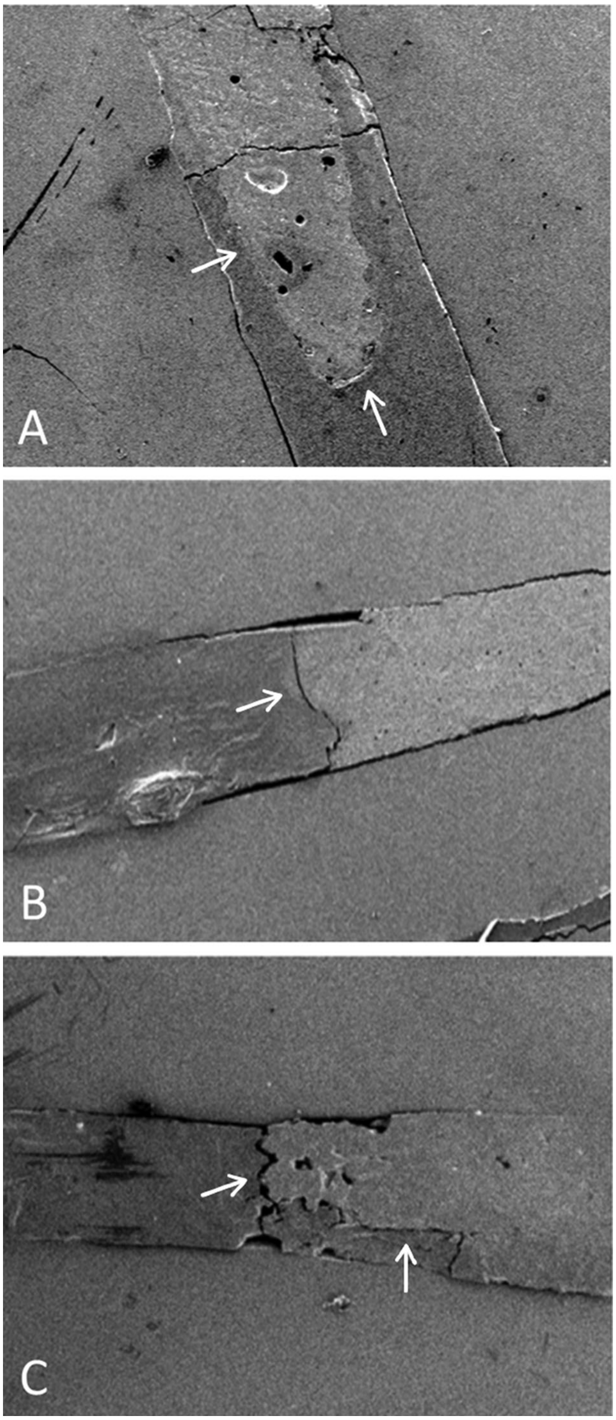

Fig 2. SEM imaging of cavities filled with the root-end filling materials. (A) Super EBA; (B) MTA; and (C) Portland cement. The narrows indicate the interface between the filling and root-end fillings materials.

of root-end cavities filled with Super EBA, Portland cement, and MTA.

Discussion

The search for a root-end material that fits properly to root-end cavity and the concern about the elimination of empty spaces within the root canal has motivated numerous studies in Endodontics. The use of SEM has been used as a valuable tool to assess the marginal adaptation of various root-end filling materials to the root dentin by using longitudinal sections of the tooth (Badr, 2010; Munhoz *et al.*, 2011; Chittoni *et al.*, 2012). The main advantage of SEM is a highly detailed observation of the evaluated area. On the other hand, this methodology presents some drawbacks; preparation of biologic samples before the SEM examination might be associated with the introduction of many artifacts (Badr, 2010). High vacuum evaporation can cause artifacts such as cracks in root dentin and separation and lifting of the filling material from the root canal walls (Badr, 2010). In addition, there might be expansion or contraction of the tooth, filling material or root-end filling material due the heating required for the preparation of specimens. Nevertheless, SEM still consists in the main methodology to evaluate the adaptation of root-end filling materials with the root dentin.

The apical fragment often is longitudinally sectioned with double-faced diamond discs (Bidar *et al.*, 2007). However, this procedure may leads to dislodgement of the gutta-percha, the endodontic sealer or the root-end filling material. Thus, the sectioning method may affect the outcome of the study (Bidar *et al.*, 2007). The force and vibration due to sectioning can cause increases in the mean gap size (Bidar *et al.*, 2007). Shipper *et al.* (2004) sectioned the specimens after blocking to avoid the material dislodgment. In the current study sectioning was performed without blocking, but to minimize this shortcoming, the specimens were initially sectioned so that the section would not reach the root canal (Fig. 1E). Next, they were sanded using water-wet sandpaper with crescent grit numbers (800, 1,200, 1,500, 2,000, and 2,500) in a polishing machine to exposure the area of interest (Chittoni *et al.*, 2012).

The results of this study accepted the hypothesis that there were statistically significant differences among the three root-end filling materials. Low amount of gaps were observed in MTA group than in Super EBA group ($p = 0.0004$). Although significant differences have been observed, the three root-end filling materials presented great adaptation to the filling material. This result agrees with previous studies in which MTA presented great adaptation to root dentin (Torabinejad *et al.*, '93; Gondim *et al.*, 2003). Torabinejad *et al.* ('93) compared the marginal adaptation of four root-end filling materials (MTA, Amalgam, Super-EBA, and IRM) and concluded that MTA presented best adaptation than Super EBA. Previous reports also agree with these findings (Xavier *et al.*, 2005; Bidar *et al.*, 2007). According to Gondim *et al.* (2003), MTA present better marginal adaptation than Super EBA irrespective of the finishing techniques for burnishing. However, up to date there is no study that evaluated the adaptation of root-end filling to filling material.

MTA and Super EBA could be used as root-end filling materials because of their favorable physicochemical and biological properties (Torabinejad *et al*., '93). Super EBA presents good cost−benefit relationships and is easy to manipulate and insert into the root-end cavity. MTA has been widely used in Endodontics, and various studies have demonstrated its good sealing capacity and biocompatibility (Lee *et al*., '93; Torabinejad *et al*., '93). Because MTA is composed primarily of Portland cement (Estrela *et al*., 2000), some authors have performed studies with it (Saidon *et al*., 2003). Despite the hard handling of MTA and Portland cement, they presented great adaptation to the filling material.

The great adaptation of the MTA to the filling material may be attributed to its physical and chemical properties. MTA powder consists of fine hydrophilic particles that absorb water during hydration. Therefore, the material expands during setting, which must have played a role in its superior adaptation to the root canal filling (Storm *et al*., 2008; Hawley *et al*., 2010). The superior adaptation of MTA has been shown in previous reports, especially when compared with amalgam, glass ionomer, and Super EBA (Torabinejad *et al*., '93; Gondim *et al*., 2003; Xavier *et al*., 2005). Portland cement and MTA did not present statistical difference in the present study. Some authors suggest that Portland cement could replace MTA in dentistry due their similar physical properties and because Portland cement is the main component of the MTA (Camilleri *et al*., 2005). However, a recent *in vivo* research showed that the biological behavior of these materials is not identical. Due the great releasing of calcium ions, MTA can improve the process of mineralization faster than Portland cement (Dreger *et al*., 2012). Moreover, MTA presents fewer amounts of toxic heavy metals and it goes through a better purification process than ordinary Portland cement (Dammaschke *et al*., 2005).

Even with the small amount of gaps observed in this study, the adaptation of root-end filling materials to the filling mass or to the root canal walls may be improved. Araújo *et al*. (2012) evaluated the marginal adaptation of MTA when used as apical barrier. Minimal linear measurements of gaps were observed when smear layer removal was performed and MTA was placed with indirect ultrasonic activation (Araújo *et al*., 2012). Thus, further researches must be carried out to evaluate the influence of ultrasonics in presence of gaps at filling and root-end filling interface and the role of ultrasonics and smear layer removal in marginal adaptation of root-end filling materials.

Conclusion

Based on the SEM images that presented small amount of gaps at the interface filling/root-end filling materials, it can be concluded that Super EBA, MTA, and Portland cement presented great adaptation to the root canal filling.

References

Al-Hezaimi K, Naghshbandi J, Oglesby S, Simon JHS, Rotstein I. 2005. Human saliva penetration of root canals obturated with two types of mineral trioxide aggregate cements. J Endod 31:453–456.

Araújo AC, Nunes E, Fonseca AA, *et al*. 2012. Influence of smear layer removal and application mode of MTA on the marginal adaptation in immature teeth: a SEM analysis. Dent Traumatol 29:212–217.

Badr AE. 2010. Marginal adaptation and cytotoxicity of bone cement compared with amalgam and mineral trioxide aggregate as root-end filling materials. J Endod 36:1056–1060.

Balto H, Al-Nazhan S. 2003. Attachment of human periodontal ligament fibroblasts to 3 different root-end filling materials: scanning electron microscope observation. Oral Surg Oral Pathol Oral Med 95:222–227.

Bernabé PEF, Holland R, Morandi R, *et al*. 2005. Comparative study of MTA and other materials in retrofilling of pulpless dogs' teeth. Braz Dent J 16:149–155.

Bidar M, Moradi S, Jafarzadeh H, Bidad S. 2007. Comparative SEM study of the marginal adaptation of white and grey MTA and Portland cement. Aust Endod J 33:2–6.

Camilleri J, Montesin FE, Brady K, *et al*. 2005. The constitution of mineral trioxide aggregate. Dent Mater 21:297–303.

Chittoni SB, Martini T, Wagner MH, *et al*. 2012. Back-scattered electron imaging for leakage analysis of four retrofilling materials. Microsc Res Tech 75:796–800.

Dammaschke T, Gerth HU, Züchner H, Schafer E. 2005. Chemical and physical surface and bulk material characterization of white ProRoot MTA and two Portland cements. Dent Mater 21:731–738.

Dreger LA, Felippe WT, Reyes-Carmona JF, *et al*. 2012. Mineral trioxide aggregate and Portland cement promote biomineralization in vivo. J Endod 38:324–329.

Estrela C, Bammann LL, Estrela CR, Silva RS, Pécora JD. 2000. Antimicrobial and chemical study of MTA, portland cement, calcium hydroxide paste, Sealapex and Dycal. Braz Dent J 11:3–9.

Gondim E, Zaia AA, Gomes BP, *et al*. 2003. Investigation of the marginal adaptation of root-end filling materials in root-end cavities prepared with ultrasonic tips. Int Endod J 36:491–499.

Hawley M, Webb TD, Goodell GG. 2010. Effect of varying water-to-powder ratios on the setting expansion of white and gray mineral trioxide aggregate. J Endod 36:1377–1379.

Johnson BR. 1999. Considerations in the selection of a root-end filling material. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 87:398–404.

Kim S, Kratchman S. 2006. Modern endodontic surgery concepts and practice: a review. J Endod 32:601–623.

Lazarski MP, Walker WA III, Flores CM, Schindler WG, Hargreaves KM. 2001. Epidemiological evaluation of the outcomes of nonsurgical root canal treatment in a large cohort of insured dental patients. J Endod 27:791–796.

Lee SJ, Monsef M, Torabinejad M. 1993. Sealing ability of a mineral trioxide aggregate for repair of lateral root perforations. J Endod 19:541–544.

Munhoz MF, Marchesan MA, Cardoso DR, *et al*. 2011. Quantitative 3D profilometry and SEM analysis of the adaptation of root-end filling materials placed under an optical microscope. Int Endod J 44:560–566.

Nair U, Ghattas S, Saber M, *et al*. 2011. A comparative evaluation of the sealing ability of 2 root-end filling materials: an in vitro

leakage study using *Enterococcus faecalis*. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 112:e74–e77.
Olsen FK, Austin BP, Walia H. 1994. Osseous reaction to implanted ZOE retrograde filling materials in the tibia of rats. J Endod 20:389–394.
Oynick J, Oynick T. 1978. A study of a new material for retrograde filling. J Endod 4:203–206.
Reyes-Carmona JF, Felippe MS, Felippe WT. 2010. A phosphate-buffered saline intracanal dressing improves the biomineralization ability of mineral trioxide aggregate apical plugs. J Endod 36:1648–1652.
Saidon J, He J, Zhu Q, Safavi K, Spangberg LS. 2003. Cell and tissue reactions to mineral trioxide aggregate and Portland cement. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 95:483–489.
Shipper G, Grossman ES, Botha AJ, Cleaton-Jones PE. 2004. Marginal adaptation of mineral trioxide aggregate (MTA) compared with amalgam as root-end filling material: a low-vacuum (LV) versus high-vacuum (HV) SEM study. Int Endod J 37:325–336.
Storm B, Eichmiller FC, Tordik PA, Goodell GG. 2008. Setting expansion of gray and white mineral trioxide aggregate and Portland cement. J Endod 34:80–82.
Torabinejad M, Watson TF, Pitt Ford TR. 1993. Sealing ability of a mineral trioxide aggregate when used as a root end filling material. J Endod 19:591–595.
Torabinejad M, Smith PW, Kettering JD, Pitt Ford TR. 1995. Comparative investigation of marginal adaptation of mineral trioxide aggregate and other commonly used root-end filling materials. J Endod 21:295–299.
Xavier CB, Weismann R, de Oliveira MG, Demarco FF, Pozza DH. 2005. Root-end filling materials: apical microleakage and marginal adaptation. J Endod 31:539–542.