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Back-Scattered Electron Imaging for Leakage Analysis of Four Retrofilling Materials

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KEY WORDS back-scattered electron imaging; endodontics; retrofilling material; leakage

ABSTRACT This study aimed to evaluate, *ex vivo*, the nanoleakage in dentinal tubules, the linear infiltration of silver nitrate in the dentin wall/root-end filling material interface, and the presence of gaps in this interface in root-end cavities filled with 4 filling materials. Forty-eight distobuccal root canals of maxillary molars were instrumented and filled. Retrograde cavities were prepared with ultrasonic points (apical 2 mm). The samples were divided into 2 control groups ($n = 4$) and 4 experimental groups ($n = 10$): Group I – white mineral trioxide aggregate (MTA); Group II – Super EBA; Group III – Portland cement; and Group IV – Sealer 26. After 1 week, the specimens were subjected to silver nitrate and prepared for SEM (backscattered electrons). In the apical-apical segment, an area with significantly higher leakage was observed for Super EBA, followed by Portland cement, MTA, and Sealer 26 ($P = 0.0054$). In the medium and cervical segments, all materials showed the same leakage behavior ($P = 0.1815$ and $P = 0.1723$, respectively). The linear infiltration at the dentin wall/root-end filling material interface was higher with Super EBA than the other groups. No differences in the percentage of gaps along the 3 mm of dentin wall/root-end filling material interface between the 4 materials were evident ($P > 0.05$). Nanoleakage occurred mainly in the apical segment of the samples, and Super EBA showed the highest values. The area and linear leakage were lower in the middle and coronal segments, regardless of the root-end filling material. No material perfectly sealed the root-end cavities, which allowed for the leakage occurrence. *Microsc. Res. Tech.* 75:796–800, 2012. © 2011 Wiley Periodicals, Inc.

INTRODUCTION

The anatomic complexity of the root canals, particularly of the apical third, is a limiting factor in preparation and root canal filling techniques. Even so, when a root canal treatment is performed with attention to biological and technical aspects, success may be achieved in ~90% of cases (Sjögren et al., 1990). Correct apical sealing will allow for tissue repair. However, in cases in which radiography shows great root canal filling with presence of a periapical lesion associated with signs and symptoms should be attempted resolution through the retreatment or periradicular surgery, with retreatment being the first choice. Endodontic surgery is an alternative to tooth extraction when endodontic retreatment is unsuccessful or impossible, because it is not possible to attain coronal access. Retrograde filling is the primary surgical modality of choice, and it involves filling up of an apical cavity. Ultrasonic root-end points were developed specifically for root-end preparation of apical cavities and have brought in a new age of endodontic surgery. Because of their reduced size and efficient cutting capacity (Dentkos and Berzins, 2008), they can be easily introduced into the bone cavity and apical foramen, allowing for long-axis preparation of root cavities, even in inaccessible areas (Carr, 1992).

Root-end filling material must have satisfactory physicochemical and biological properties. The physicochemical properties include great sealing ability. To test the sealing ability of root-end filling materials, studies have used bacterial infiltration (Maltezos et al., 2006; Montellano et al., 2006), penetration of human saliva (Al-Hezaimi et al., 2005), dye leakage (Jenkins et al., 2006), microleakage of bovine serum albumin (Saghiri et al., 2008), and fluid infiltration (Yildirim et al., 2008).

Among root-end filling materials, mineral trioxide aggregate (MTA) was developed to aid the sealing of communications between the teeth and the external surface (Lee et al., 1993). MTA has satisfactory sealing capacity as a material used for retrofilling (Al-Hezaimi et al., 2005; Aqrabawi, 2000; Torabinejad et al., 1995), promoting favorable tissue repair response (Bernabé et al., 2005; Tanomaru-Filho et al., 2006; Tawil et al., 2009; Torabinejad et al., 1997). Another root-end filling material is Super EBA. It contains aluminum oxide

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Received 5 October 2011; accepted in revised form 1 November 2011

DOI 10.1002/jemt.21128

Published online 6 December 2011 in Wiley Online Library (wileyonlinelibrary.com).

and the orthoethoxybenzoic acid, which reduce eugenol and make this material more biologically compatible with periapical tissues. Sealer 26 is a material based on epoxy resin and contains calcium hydroxide. It showed satisfactory biological properties in a study involving retrograde fillings in dogs' teeth with periapical injury (Tanomaru-Filho et al., 2006). The increased powder/liquid proportion of this sealer results in a greater consistency, allowing for its insertion into retrocavities (Tanomaru-Filho et al., 2006).

Recently, several articles on adhesive restorative dentistry have used nanoleakage to explain the behavior of different materials at the resin–dentin interface (Babb et al., 2009; Dündar et al., 2011; Hiraishi et al., 2009; Klein-Júnior et al., 2008; Makishi et al., 2010; Navarra et al., 2009; Roberts et al., 2009; Tay et al., 2002). However, few studies have examined the sealing ability of retrograde filling materials in terms of nanoleakage. Therefore, the aim of this study is to evaluate, *ex vivo*, the nanoleakage at the dentin-filling material interface at the root-end area using four different cements, testing the hypothesis that there is no difference in the sealing ability of the cement materials evaluated.

MATERIALS AND METHODS

Selection, Preparation, and Root Canal Fillings

This study was submitted and approved by Ethical Committee of the Rio Grande do Sul Federal University. Forty-eight disto-buccal roots of extracted human first molars were placed in 0.9% saline after storage in 2% hypochlorite sodium solution for 48 h. The specimens were transversely sectioned at the cement–enamel junction with a diamond disc under water cooling. Tooth length was established by a #15 K-file (Dentsply-Maillefer, Ballaigues, Switzerland) at 1 mm from the apex, and root canal preparation was performed to the #40 K-file and irrigation with 1% sodium hypochlorite solution after each instrumental change. The root canals were dried and filled with #40 principal gutta-percha points and B7 accessories gutta-percha cones (Tanariman Indústria Ltda., Manacapuru, Brazil), and Endofill (Dentsply-Maillefer, Petrópolis, Brazil) using cold lateral compaction technique.

Cavity Preparation and Retrograde Filling

Twenty-four hours after filling, the specimens were subjected to apicoectomy. The Endo-Z bur (Dentsply Indústria e Comércio Ltda, Petrópolis, Brazil) was positioned perpendicular to the long axis of the root, 2-mm short of the apex. Retrograde cavities 3-mm deep were subsequently prepared using ultrasound (CVDent 1000; São José dos Campos, Brazil) and ultrasound tips (TOP-2, CVDentus; São José dos Campos, Brazil) with continuous irrigation of saline solution. The roots were randomly divided into four experimental groups and two control groups, 10 and four specimens each, respectively.

The retrograde cavities were filled with: Portland cement (Votorantin, São Paulo, Brazil), white MTA (Angelus Indústria de Produtos Odontológicos S/A, Paraná, Brazil), Super EBA (Harry J. Bosworth Co., Skokie), or Sealer 26 (Dentsply Indústria e Comércio Ltda, Petrópolis, Brazil). Eight tooth roots were used as control, four did not receive filling material (positive

controls), and the remaining four were coated with cyanoacrylate (Loctite Super Bonder, Brazil) after retrograde filling using one experimental material in each root canal (negative control). Super EBA was manipulated to a powder/liquid proportion of 1 g per 0.2 mL (Bernabé et al., 2005). Portland cement and white MTA were manipulated according to the manufacturers' instructions, and Sealer 26 was manipulated by using a greater amount of powder, forming a thicker consistency with a powder/liquid proportion of 5:1 (Tanomaru-Filho et al., 2006). To set the materials, they were placed inside a covered plastic container containing gauze soaked in distilled water for 1 week at 37°C.

Scanning Electron Microscopy

A layer of insulating varnish was applied to the outer surface of the roots. All specimens were subsequently immersed in 1 L of ammoniacal silver nitrate solution and distilled water (equal parts) for 24 h and kept in the dark. The specimens were then copiously washed in running water for 1 h and submerged for 8 h in developing solution (Kodak, Rochester). After this protocol, each specimen was worn longitudinally with 800, 1,200, 1,500, 2,000, and 2,500 water-wet sandpaper (3M, St. Paul) in a polishing machine (Buehler, Lake Bluff), and final polishing was performed with 1/4- μ m granulation paste (Arotec, São Paulo, Brazil). The specimens were subjected to slow dehydration with silica gel, mounted onto specific stubs for observation under the JEOL 5800 scanning electronic microscope (SEM) (JEOL, Tokyo, Japan) and coated only with carbon coverage (MED 010; Balzers Union, Balzers, Liechtenstein, Germany). This SEM is equipped with detectors for electron backscatter images (backscattered electrons).

Nanoleakage Analysis

To analyze the silver nitrate leakage capacity against tested root-end filling materials, images taken with the SEM were transferred to on ImageTool 3.0 (UTHSCSA, San Antonio). First, images with a panoramic view and with $\sim 30\times$ magnification were used. All images were presented on a micrometer scale, which was essential for calibration of software measurement tools. The calibration tool was selected, and a trace was made exactly on the scale bar. The number of pixels that the scale represented in micrometers was obtained. A window then opened, into which was placed the millimeter value corresponding to the length of the bar in micrometers. Software tools were then calibrated to obtain the values in millimeters.

Marker penetration was performed in a linear manner, considering as measurement a line drawn on the entire material/dentin wall interface where the dye penetrated, from the apex of the material until its cervical portion on both sides. For the statistical analysis, the longest line was considered. To determine the leakage extent, values were automatically provided in millimeters by the software. Another group of images, which had a $500\times$ magnification, was then measured. The dentin stained area was measured and compared with total dentin area. Both measurements were provided in square millimeters, and the values were transformed into percentage of stained area. The same measurement protocol was conducted to evaluate the gap areas.

TABLE 1. Percentage (%) of dentin area infiltrated by silver nitrate

	Portland <i>n</i> (median)	MTA <i>n</i> (median)	Sealer 26 <i>n</i> (median)	Super EBA <i>n</i> (median)
Cervical–apical	10 (2.72 ^a)	10 (5.76 ^a)	10 (1.64 ^a)	9 (0.75 ^a)
Middle–apical	10 (2.41 ^a)	10 (5.29 ^a)	10 (4.02 ^a)	9 (2.98 ^a)
Apical–apical	10 (3.37 ^a)	10 (11.27 ^{ab})	10 (6.56 ^{ab})	9 (20.53 ^b)

Same letters in the same line do not differ statistically by Kruskal–Wallis test and post hoc Dunn. Significance level of 5%.

All measurements were performed by a single observer and were properly calibrated. Each measurement was performed five times at intervals of 48 h.

The data for the percentage area of dentin infiltrated, linear leakage, and percentage of linear gaps were subject to the Kruskal–Wallis and post hoc Dunn tests. Comparison between groups with respect to num-

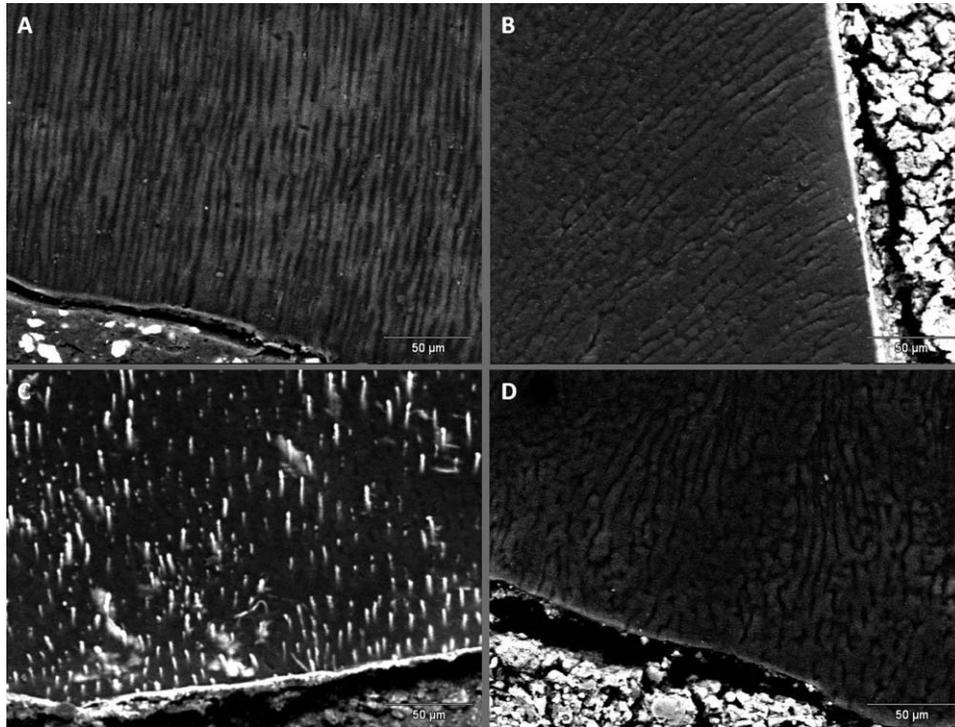


Fig. 1. Nanoleakage into dentinal tubules of root-end cavities filled with Sealer 26 (A) and white MTA (B) in apical–cervical segment and with Super EBA (C) and Portland cement (D) in apical–apical segment.

TABLE 2. Median of linear leakage (in millimeters) at the dentin wall/retrofilling material interface

Portland <i>n</i> (median)	MTA <i>n</i> (median)	Sealer 26 <i>n</i> (median)	Super EBA <i>n</i> (median)
10 0.00 ^a	10 (0.43 ^a)	10 (0.10 ^a)	9 (1.17 ^a)

Same letters in the same line do not differ statistically by Kruskal–Wallis test. Significance level of 5%.

TABLE 3. Percentage (%) of teeth with nanoleakage

	With nanoleakage	Without nanoleakage	Total
Sealer 26	50 ^a	50	100
EBA	62.50 ^a	37.59	100
MTA	90.91 ^a	9.09	100
Portland	44.44 ^a	55.56	100

Same letters in the same column do not differ statistically by chi-square test. Significance level of 5%.

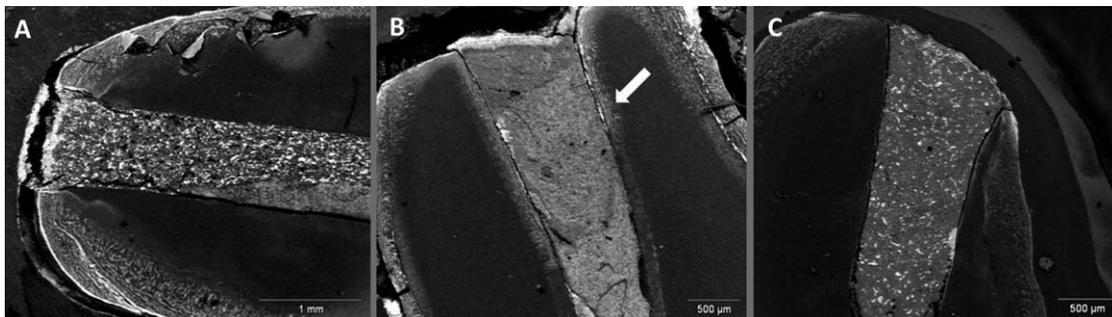


Fig. 2. Back-scattered electron imaging of root-end cavities filled with (A) Sealer 26; (B) Super EBA; and (C) MTA. The arrow (B) indicates linear nanoleakage in the apical 3 mm.

TABLE 4. Mean and standard deviation of the percentage (%) of gaps along the 3 mm of dentin / root-end filling material interface

	Portland	MTA	Sealer 26	Super EBA
Apical–apical	5.077 ^a 4.178	6.484 ^a 5.384	5.213 ^a 4.297	3.411 ^a 3.399
Middle–apical	3.588 ^a 3.151	2.968 ^a 2.477	2.598 ^a 1.708	2.623 ^a 2.002
Cervical–apical	2.353 ^a 1.823	4.760 ^a 3.144	2.086 ^a 0.996	3.629 ^a 2.826

Same letters in the same line do not differ statistically by Kruskal–Wallis test. Significance level of 5%.

ber of teeth with nanoleakage was performed using the chi-square test with a significance level of 5%.

RESULTS

The negative control group showed no impregnation of silver nitrate, while the positive control specimens showed a median percentage area of infiltration of 94.73%. A sample group of Super EBA was lost during processing for SEM.

Table 1 shows the median percentage of infiltrated dentin analyzed in three segments: apical–apical, apical–middle, and apical–cervical. A statistically significant difference ($P < 0.05$) was observed between the apical–apical segments of the Portland cement and Super EBA groups. The apical–middle and apical–cervical segments of the four experimental groups showed similar marker infiltration ($P > 0.05$). The nanoleakage of root-end cavities in apical–cervical and apical–apical segment is presented in Figure 1.

Table 2 summarizes the linear leakage median of the groups. In this evaluation, the highest values were recorded for Super EBA and the lowest for Portland cement but without significant differences ($P > 0.05$) in comparisons of the studied materials. Figure 2 presents back-scattered electron imaging of root-end cavities filled with Sealer 26; Super EBA; and MTA.

Table 3 describes the percentages of teeth with and without nanoleakage in the four tested groups. There were no statistically significant differences among the tested materials ($P > 0.05$). The values shown in Table 4 demonstrate no differences between the four root-end sealers in the percentage of gaps along the 3 mm of dentin/root-end filling material interface ($P > 0.05$).

DISCUSSION

For successful retrograde fillings, it is essential to use a material that provides appropriate sealing conditions, minimizing the consequences of marginal leakage. Marginal leakage studies have been conducted with human saliva (Al-Hezaimi et al., 2005), fluid filtration (Yildirim et al., 2008), and dye solutions such as India ink (Jenkins et al., 2006) and blue methylene (Aqrabawi, 2000). However, the use of dyes has been questioned to evaluate the marginal leakage, because the sealing capacity of the material may be influenced by the dye solution, producing results that are not reproducible and that have high standard deviations (Editorial Board of the Journal of Endodontics, 2007; Tanomaru-Filho et al., 2005; Wu et al., 1998).

MTA, Super EBA, and Sealer 26 are three available materials that could be used as root-end filling materials because of their favorable physicochemical and biological properties (Torabinejad et al., 1995, 1997). Sealer 26 and Super EBA have good cost-benefit relationships and are 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., 1993; Torabinejad et al., 1995, 1997). However, this material has the disadvantage of being difficult to manipulate. Because MTA is composed primarily of Portland cement (Estrela et al., 2000), some authors have conducted studies with this material (Saidon et al., 2003), which motivated its use in the present study.

The use of ultrasonic root-end points made it possible to produce conservative preparations with parallel walls ~3-mm deep (Carr, 1992) and allowed for the analysis of every millimeter of the material/canal wall interface.

Nanoleakage was originally used to describe micro-pore zones under or in the hybrid layer in studies of tooth/resin interface conditioned by self-etching primers (Sano et al., 1995; Tay et al., 2002). Nanoleakage verified the penetration of a marker in the absence of interfacial gaps (Fischer et al., 1998). However, this technology has been insufficiently explored to evaluate the sealing capacity of root-end filling materials.

The results obtained in this study demonstrated that statistically significant differences were found for the most apical millimeter (apical–apical). The percentages of dentin areas infiltrated by the marker differed between Super EBA and Portland cement. A probable explanation is that Super EBA is a soluble and a technique-sensitive material, which promotes greater penetration of the marker. In the remaining 2 mm (apical–medium and apical–cervical), no differences between the four studied groups were observed.

Nanoleakage in retrofilling materials was not reported. In terms of bacterial leakage, other studies found that Super EBA had results superior to those of MTA (Aqrabawi, 2000; Maltezos et al., 2006). However, in histopathologic analysis, the results of MTA and Super EBA were similar (Bernabé et al., 2005). The few studies that involve the use of Sealer 26 as a root-end filling material are in vivo studies with histopathological findings similar to those obtained with the use of MTA (Tanomaru-Filho et al., 2006). De-Deus et al. (2007) found no differences between MTA and Portland cement in the sealing of furcal perforations using the fluid filtration method and leakage occurred in all samples of the experimental groups. In the present study, using the same materials as those in root-end filling, nanoleakage did not show statistically significant differences between these two materials; however, several specimens without infiltration were observed for both materials.

Evaluation of the linear leakage of the marker revealed no differences in the material/dentin wall interface between the four materials studied; however, the highest mean values were calculated for the Super EBA group, confirming the percentage of area with nanoleakage for the same material. It is important to consider that there was no difference between the materials in terms of the number of teeth with nanoleakage; no group showed perfect sealing of the root-end cavities for any of the specimens.

Excellent sealing capacity of the tested materials was also shown by the low gap percentages (~3%–6.5%) in the four groups; no significant differences were observed between them. These results validate the use of MTA, Super EBA, and Sealer 26 as retrofilling materials. Despite satisfactory results, further studies on Portland cement are needed so that it can be used in clinical practice.

No material perfectly sealed the root-end cavities, which allowed for the nanoleakage occurrence. The largest percentage of nanoleakage and linear infiltration areas by the marker occurred in the first millimeter of the cavities (apical–apical), with higher values for Super EBA. The percentage of area linearly infiltrated by the marker was small and similar for the four retrofilling materials in the two more cervical millimeters of the root-end cavities.

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