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Quantitative 3D profilometry and SEM analysis of the adaptation of root-end filling materials placed under an optical microscope

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Abstract

Munhoz MF, Marchesan MA, Cardoso DRF, Silva SRC, Silva-Sousa YTC, Sousa-Neto MD. Quantitative 3D profilometry and SEM analysis of the adaptation of root-end filling materials placed under an optical microscope. *International Endodontic Journal*, **44**, 560–566, 2011.

Aim To evaluate by 3D profilometry and scanning electron microscopy (SEM), the marginal adaptation of mineral trioxide aggregate (MTA) and Sealer 26 placed in root-end cavities with direct vision or under an optical microscope.

Methodology The root ends of 52 root filled canine teeth were filled with MTA or Sealer 26 under direct vision or optical microscope ($n = 13$). In each group, eight specimens were analysed by profilometry for measurement of the area and depth of gaps. In the other five specimens, gap area was measured using SEM to verify marginal adaptation and surface characteristic. Data were analysed by parametric (ANOVA and Tukey) and non-parametric (Kruskal–Wallis and Dunn) tests.

Results The assessment of the adaptation of both materials to dentine was not influenced by the mode of visualization, which was confirmed by both profilometry and SEM observations. The voids measured with profilometry for Sealer 26 under direct vision were significantly wider and deeper than those for MTA under direct vision ($P < 0.05$). In SEM, significantly larger gap areas were observed with Sealer 26 ($P < 0.05$).

Conclusion Root-end cavities filled with MTA had smaller gaps and better marginal adaptation than Sealer 26.

Keywords: profilometry and endodontics, root-end filling materials, SEM.

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Introduction

The clinical use of optical microscopy in Endodontics enhances visibility of the periradicular region by providing greater illumination and magnification (Rubinstein 2005, Carr & Murgel 2010). This enhances the identification of anatomical structures, as well as root cracks, fractures and perforations, and allows refinement of operative procedures such as root-end cavity preparation and root-end filling (Kersten *et al.* 2007).

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The root-end filling material is another important factor to be considered for a successful endodontic therapy because it remains in intimate contact with the periradicular tissues (Torabinejad *et al.* 1993, Siqueira *et al.* 2001, Maltezos *et al.* 2006, Tanomaru-Filho *et al.* 2006, Ferk Luketić *et al.* 2008). The quality of the root-end filling and its adaptation to the cavity walls along with the apical seal have been evaluated by scanning electron microscopy (SEM) (Lloyd *et al.* 1996, Peters & Peters 2002, Asgary *et al.* 2009), marginal leakage assays with different dyes (Vogt *et al.* 2006, Ferk Luketić *et al.* 2008) and bacterial leakage studies (Siqueira *et al.* 2001, Maltezos *et al.* 2006). Attin *et al.* (2009) used three-dimensional (3D) profilometry to evaluate the influence of drying eroded dentine and enamel surfaces and observed that this method provided high-resolution images and accurate analysis.

Therefore, the aim of this study was to evaluate the marginal adaptation of root-end filling materials placed in root-end cavities with direct vision or under an optical microscope by means of 3D profilometry and SEM analyses. The null hypothesis is that root-end fillings performed under optical microscope will not be adapted better than those carried out under direct vision.

Materials and methods

This study was approved by the Research Ethics Committee of the University of Ribeirão Preto, São Paulo, Brazil.

Fifty-two extracted human maxillary canine teeth with complete root formation, single canal, no internal resorptions or calcifications, and no accentuated root curvatures were selected.

After access, a size 15 K-file (Dentsply Maillefer, Ballaigues, Switzerland) was introduced into the canal until the apical foramen. The working length was established by subtracting 1.0 mm from this measurement. Root canals were prepared using manual K-files (Dentsply Maillefer) until size 40. Canals were irrigated with 2 mL of 1% NaOCl between files. At the end of instrumentation, canals were flooded with 2 mL of 17% EDTA for 5 min. Final irrigation was achieved with 10 mL of distilled water.

Approximately, 3 mm of the root ends were resected as perpendicular as possible to the long axis of the tooth with a number 151 Zecrya bur (Dentsply Maillefer) at high speed and under abundant water cooling. Root-end cavities of 3 mm depth were prepared with 12/90 ultrasound-activated diamond tips (Trinity, São Paulo, SP, Brazil) attached to a piezoelectric ultrasonic unit (Gnatus, Ribeirão Preto, SP, Brazil) with coupled peristaltic pump at 29 kHz frequency under saline cooling. After root-end cavity preparation, the teeth were decoronated at the cementoenamel junction with a water-cooled double-faced diamond disc (KG Sorensen, Barueri, SP, Brazil) and 52 root segments were obtained.

Root-end resection and cavity preparation of all teeth were carried out under an OM (DF Vasconcelos, São Paulo, SP, Brazil). The root-end cavities were filled with either a resin-based sealer (Sealer 26; Dentsply Ind. e Com. Ltda., Petrópolis, RJ, Brazil) or white mineral trioxide aggregate (MTA) (Angelus, Londrina, PR, Brazil) under direct vision or optical microscope, thus providing four groups of 13 specimens each: MTA with direct vision, MTA under optical microscope, Sealer 26 with direct vision and Sealer 26 under optical microscope.

For preparation of the root-end cavities, an autopolymerizing acrylic resin device with a trapezoidal design was fabricated to simulate the limited access to the root-end during apical surgery. This device had two openings: an 8-mm diameter opening on the centre of its base for insertion and fixation of the root segment and a 5-mm diameter opening on one of its lateral walls simulating the bone cavity through which the root-end is reached for root-end cavity preparation (Fig. 1).

Sealer 26 was prepared by progressively incorporating the powder to the liquid until a thick paste was obtained, which was rolled into a cylindrical shape and placed in the root-end cavity in a single increment using a surgical microspatula. For white MTA, the powder was progressively incorporated into distilled water to produce a consistent paste, which was taken to the root-end cavity in increments using a surgical microspatula, followed by burnishing with a microburnisher. The specimens were maintained at 37 °C and 95% relative humidity for 36 h to allow complete setting of the sealers.

Profilometry

Eight specimens of each group were examined with a 3D profilometer (Talysurf CLI 1000; Taylor Hobson, Leicester, UK). The surface 3D profilometry data (vertical variation along the surface), expressed in μm, were recorded and processed with the Talymap Analysis software that is supplied with the equipment. Taylor This software generates colour axonometric images of the surface, which are accompanied by a scale in which each colour represents a depth level. These images were used for the analysis of linear,

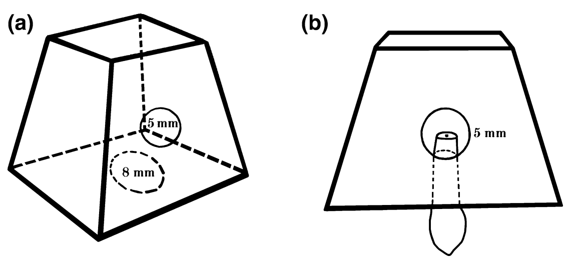

Figure 1 Schematic drawing of the acrylic device fabricated to simulate the bone cavity. Lateral view showing the 8-mm-diameter central opening (a); Cavity with the specimen (b).

superficial and 3D measurements of the topographical variations. Using the Talymap Analysis software, the axonometric images of each specimen were analysed in eight standardized and equidistant points that resembled the compass wind rose with its four cardinal directions (N, E, S, W) and four intermediate directions (NE, SE, SW, NW). Black and white images obtained from saturated original colour 3D images were used to obtain a better visualization of the extension of the interface between the root-end cavity and the root-end filling material and to check for gaps in this region (Fig. 2). When gaps were found, the following measurements were obtained using the software: *gap area* (in μm^2), which was delimited by demarcating one point at the beginning of the root-end cavity margin and another point at the highest limit of the material; *maximum gap depth* (μm), which was calculated by measuring the vertical distance from the deepest point of the root-end filling material to the root-end cavity margin.

Scanning electron microscopy

Five specimens of each group were prepared for SEM analysis. To ensure a well-dried sample, specimens were stored at 37 °C for 48 h and then fixed on stubs with double-faced carbon tape (3M, São Paulo, Brazil), covered with a 30-μm gold–platinum layer (SCD 005; Bal-Tec, Zurich, Switzerland) in a vacuum apparatus (SDC 050; Baltec, Balzers, Liechtenstein). Specimens were examined with a scanning electron microscope (JEOL JSM model 5410; Jeol Technic, Tokyo, Japan), operating at 15 kV. Initially, the specimens were analysed in a panoramic vision, and then, photomicrographs of the most representative area of each group were obtained at 50 and 150 magnifications.

SEM images were analysed qualitatively by three calibrated examiners in a double-blinded manner using the UTHSCSA Image Tool software (USA). The examiners repeated the qualitative analysis after 2 weeks. The criteria observed were marginal adaptation, surface aspect and lack or excess of material. For standardization of the unit of area, the spatial measure was calibrated by drawing a 43.00-pixel line, which was converted to into millimetres (mm) by the software. The total area of the root-end cavity (ΔR) and the area occupied by the root-end filling material (ΔM) were measured. The difference between the values (ΔR − ΔM) was considered as the measurement of the projection of the gap area.

Statistical analysis

Preliminary statistical tests using GraphPad InStat software (GraphPad Software Inc., San Diego, CA, USA) were carried out to verify the normality and homogeneity of each sample. Data were not normally distributed for the variable *gap area* (μm^2) (measured in

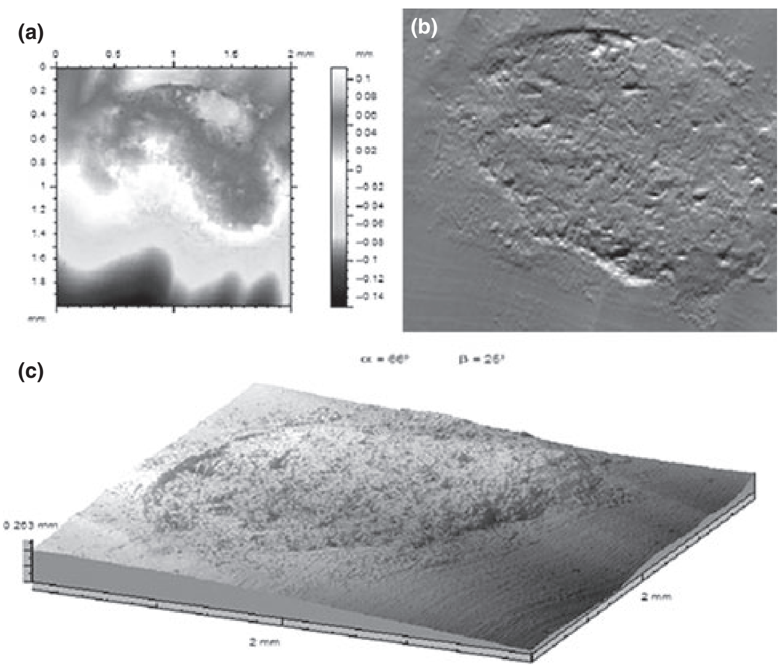

Figure 2 Images of the Talymap Analysis software. (a) Axonometric image of specimen using depth level scale; (b) Saturated image of (a) to obtain a better visualization of gap areas; (c) Saturated 3D image.

profilometry); thus, the non-parametric Kruskal–Wallis and Dunn's tests were used. For the other variables *maximum gap depth* (μm) (also measured in profilometry) and *gap value* (mm) (obtained from the SEM images), the analysis of variance (ANOVA) and Tukey's test were used because samples distributions were normal and homogeneous. A significance level of 5% was set for all analyses. Cohen's kappa coefficient was used to measure inter- and intra-examiner agreement, using the criteria proposed by Landis & Koch (1977).

Results

The inter- and intra-examiner reproducibility ranged from substantial (0.61–0.80) to excellent (0.81–1.0) (Landis & Koch 1977). The Kappa values for inter-examiners were as follows: 0.86, between A and B examiners; 0.75, between A and C examiners and 0.68, between B and C examiners. The Kappa values for intra-examiners were 0.67, between A and A examiners; 0.78, between B and B examiners and 0.84, between C and C examiners.

The 3D profilometry analysis provided the mean values of area (μm^2) and maximum depth (μm) of the gaps formed between the materials and the cavity walls in the specimens filled with MTA and Sealer 26 with direct vision and under optical microscope.

There was significant statistical difference (P = 0.0023) between the gaps observed in the MTA group with direct vision and both groups filled with Sealer 26. Larger marginal gaps were observed in the root-end cavities filled with Sealer 26 (Table 1).

There was a significant statistical difference (P = 0.003) between the maximum gap depth observed in the group filled with Sealer 26 with direct vision and both groups filled with MTA. Greater maximum depth values were observed in the root-end cavities filled with Sealer 26 (Table 2).

The SEM analysis allowed the evaluation of the surface aspect of the specimens and the dentine/material interface. The root-end filling materials differed significantly (P = 0.0000) from each other regardless of the use of a magnification device, with Sealer 26 had larger gaps in both conditions (Table 3). The analysis of the 3D profilometry and SEM images allowed the evaluation of the surface of the specimens and the dentine/material interface (Figs 3 and 4).

In the group filled with MTA with direct vision, there was good marginal adaptation of the sealer at the interface with dentine; however, some gaps were present. The group filled with MTA under optical microscope had a similar pattern of marginal adaptation with some gaps between the retrofilling material and the cavity walls, and a concave material surface. This could be clearly observed in the 3D axonometric scans that had concentric images from the centre of the retrofilling material.

The group filled with Sealer 26 with direct vision had large gaps between the material and the root-end cavity walls in some areas, whilst other areas were gap free with the material superposed on the cavity

Table 1 Gap areas (in μm^2), measured in a 3D profilometry, for the root-end filling materials placed with direct and under an optical microscope

Sealers	Median	Minimum	Maximum
MTA/direct vision	408.13^{a}	164.60	740.48
MTA/OM	407.60^{ab}	320.60	661.30
Sealer 26/direct vision	1164.90^{b}	652.33	2170.70
Sealer 26/OM	1759.90^{b}	515.17	1924.40

MTA, mineral trioxide aggregate; OM, optical microscope. Different superscript alphabets indicate statistically significant difference (Dunn's test, $P < 0.05$).

Table 2 Gap depth (in μm) for the root-end filling materials placed with direct and under an optical microscope, measured in a 3D profilometry

Sealer	Mean	Standard deviation	Minimum	Maximum
MTA/direct vision	19.05^{a}	9.62	8.06	33.00
MTA/OM	14.57^{a}	1.66	13.47	17.46
Sealer 26/direct vision	27.46^{b}	6.38	21.60	29.04
Sealer 26/OM	22.71^{ab}	7.07	16.60	32.58

MTA, mineral trioxide aggregate; OM, optical microscope. Different superscript alphabets indicate statistically significant difference (Tukey test, $P < 0.05$).

Table 3 Mean gap areas (in μm^2) for the root-end filling materials placed with direct and under an optical microscope, obtained from the SEM images

Sealer	Mean	Standard deviation	Minimum	Maximum
MTA/direct vision	120.23^{a}	5.35	112.00	124.72
MTA/OM	139.52^{a}	67.49	58.83	200.67
Sealer 26/direct vision	641.94^{b}	97.06	504.70	775.50
Sealer 26/OM	514.04^{b}	152.92	259.75	653.87

MTA, mineral trioxide aggregate; OM, optical microscope. Different superscript alphabets indicate statistically significant difference (Tukey's test, $P < 0.05$).

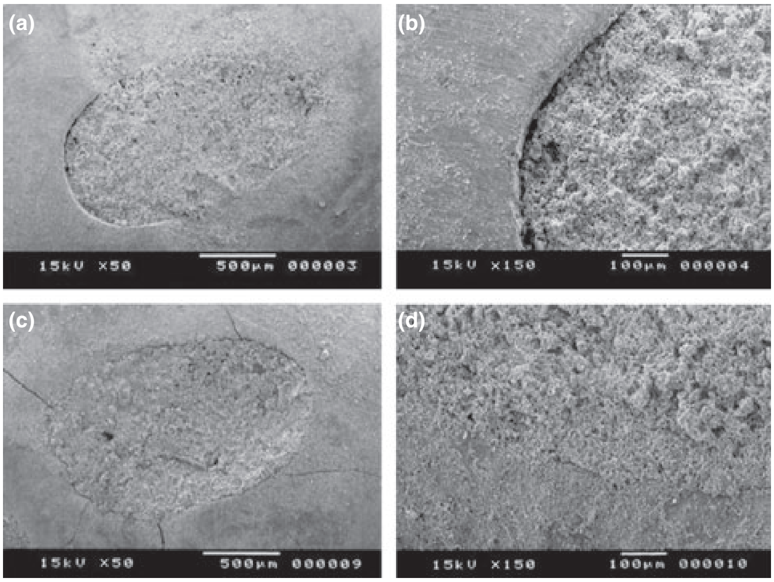

Figure 3 Representative SEM images of specimens retrofilled with mineral trioxide aggregate. (a and b) under direct vision (×50 and ×150, respectively); (c and d) under optical microscope (×50 and ×150, respectively).

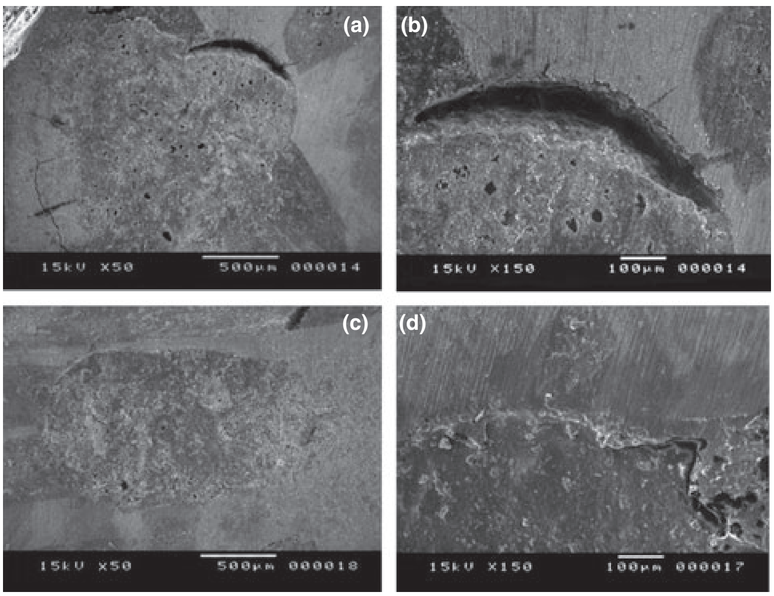

Figure 4 Representative SEM images of specimens retrofilled with Sealer 26. (a and b) under direct vision (×50 and ×150, respectively); (c and d) under optical microscope (×50 and ×150, respectively).

margins. The group filled with Sealer 26 under optical microscope had a large number of small gaps and areas with good marginal adaptation of the material, but with material superposed on the cavity margins.

Discussion

The goal of the present study was to assess, by 3D profilometry and SEM, the marginal adaptation of root-end filling materials placed in root-end cavities with direct vision or under an optical microscope.

In all specimens, root-end resection and root-end cavity preparation were performed with indirect vision under an optical microscope. The use of optical microscopy in these procedures increases the illumination and the magnification of the operative field, facilitating the detection of anatomical details that are not perceived by the naked eye (Rubinstein 2005, Kersten *et al.* 2007). Optical microscopy also contributes to the diagnosis and refinement of operative techniques (Rubinstein 2005, Kersten *et al.* 2007, Carr & Murgel 2010).

In the present study, the root-end cavities were prepared with ultrasonic tips activated by a piezoelectric ultrasonic unit. To simulate the limited access to the root-end in the bones cavity during apical surgery, an acrylic device with a central hole was used for fixation of the specimens during the operative procedures.

The use of 3D profilometry has gained popularity in dentistry for the evaluation of restorative procedures (Wilder *et al.* 2000) and eroded dental surfaces (Attin *et al.* 2009). In Endodontics specifically, this methodology has been used to assess the effects of different irrigant solution on the ultrastructural characteristics of MTA (Smith *et al.* 2007). No previous investigations have evaluated the gap formation between the root-end filling material and root-end cavity walls using 3D profilometry. This could be relevant because it provides a three-dimensional high-resolution evaluation of the sectioned areas in different planes and hence ensures more accurate measurements. Moreover, laboratory tests can be used as a screening mechanism for predicting clinical performance.

Regarding the mode of visualization for filling of root-end cavities, the findings of the present study revealed no significant difference between direct vision and the optical microscope assisted vision, when specimens filled with the same material were compared quantitatively by SEM and 3D profilometry. These results were confirmed by the qualitative analysis.

Although it has been demonstrated that the use of an optical microscope increases the illumination and magnification of the operative field (Kersten *et al.* 2007, Carr & Murgel 2010), in the present study, these advantages did not result in a better adaptation of the material to the root-end cavity. This is probably because marginal adaptation is more likely related to the physical–chemical properties of the material, such as flow, consistency and setting time, rather than the operator's visual acuity.

Comparing the materials, the results of the present study showed that the area and depth of the gaps observed at the apical dentine/retrofilling material interface were significantly larger for Sealer 26 than for MTA. This difference might be attributed to the different consistencies of the freshly prepared materials and how they were placed in the cavities. MTA is a mixture of oxides with fine hydrophilic particles that hardens in contact with water (Peters & Peters 2002, Song *et al.* 2006, Asgary *et al.* 2009). In the present study, MTA was taken to the specimens in increments that were carefully placed in to the cavity using a surgical microspatula and were then burnished with a microburnisher, which seem to have resulted in better marginal adaptation.

Sealer 26 is a resin-based sealer composed of a powder with bismuth oxide and calcium hydroxide and a paste based on bisphenol epoxy resin (Tanomaru-Filho *et al.* 2006). The thicker consistency of this sealer for root-end filling allows a single-increment insertion technique, with the material rolled into a cylindrical shape and placed into the cavity using a surgical microspatula. It is likely that this technique caused the formation of gaps in some areas and the superposition of the material onto the cavity margins in other areas. Sealers with adequate flow can penetrate and fill irregularities of the root canal system (Bernardes *et al.* 2010). The greater amount of powder incorporated to the liquid in the present study resulted in lower flow and might have contributed to the formation of gaps at the margins of the root-end cavity. Besides, drying procedures required for SEM analysis may cause shrinkage of the specimens, which can contribute to the formation of the gaps.

The null hypothesis of this study could not be confirmed because the adaptation of MTA and Sealer 26 to the margins of the root-end cavities was not influenced by the visualization of the root ends under an optical microscope. Root-end cavities filled with MTA had smaller gaps and better marginal adaptation in both 3D profilometry and SEM analysis.

Conclusion

Under the conditions of this laboratory study, the adaptation of MTA and Sealer 26 to the root-end cavities was not influenced by the mode of visualization.

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