

Micro-CT analysis of filling ability and porosity of root-end filling materials

Análise em micro-CT da capacidade de preenchimento e porosidade de materiais retrobturadores

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Resumo

Introdução: Capacidade de preenchimento da cavidade retrógrada e porosidade são propriedades importantes de materiais retrobturadores e podem ser avaliadas por meio de microtomografia computadorizada (micro-CT). **Objetivo:** Avaliar a capacidade de preenchimento e porosidade de materiais retrobturadores por meio de micro-CT. **Material e método:** Cavidades com 1 mm de diâmetro e 3 mm de altura foram preparadas em dentina bovina utilizando pontas ultrassônicas (CVD No. 6.1107-6) e foram preenchidas com Mineral Trióxido Agregado (MTA), Sealer 26 (S26) e cimento de óxido de zinco e eugenol (OZE). As cavidades foram escaneadas em micro-CT antes e após o preenchimento. A capacidade de preenchimento foi calculada com base na porcentagem em volume, das cavidades preenchidas. O número e porcentagem dos poros fechados foram avaliados em toda extensão da cavidade preenchida (total) e por terços (cervical, médio e apical) por meio de análises bi e tridimensionais. Os dados de preenchimento foram submetidos aos testes estatísticos ANOVA e Tukey e a porosidade aos testes de Kruskall-Wallis e Dunn, com nível de significância de 5%. **Resultado:** S26 e OZE apresentaram maior capacidade de preenchimento que o MTA ($p<0,05$). S26 mostrou maior porosidade total (em número e porcentagem) ($p<0,05$). Em todos os terços, após as análises 2D e 3D, a porosidade foi maior para S26 em comparação ao MTA e OZE ($p<0,05$). **Conclusão:** Embora Sealer 26 tenha apresentado maior porosidade, o material foi associado a uma adequada capacidade de preenchimento. A análise em micro-CT mostrou ausência de correlação entre capacidade de preenchimento e porosidade.

Descritores: Microtomografia por Raio-X; porosidade; materiais dentários; endodontia.

Abstract

Introduction: Filling ability of retrograde cavity and porosity are important properties for root-end filling materials and may be evaluated by using microcomputed tomography (micro-CT). **Objective:** To evaluate filling ability and porosity of root-end filling materials using microcomputed tomography (micro-CT). **Material and method:** Cavities with 1 mm internal diameter and 3 mm depth were prepared in bovine dentin sections by using ultrasonic tips (CVD No. 6.1107-6), and filled by Mineral Trioxide Aggregate (MTA); Sealer 26 (S26) and zinc oxide and eugenol cement (ZOE). Before and after filling, cavities were scanned by using micro-CT (SkyScan 1176). Filling and porosity were analyzed by using CTAn software. Filling ability was calculated based on volumetric percentage of the filled cavity. The number and percentage of closed pores were measured throughout entire extension of the filled cavity (total) and in each third (cervical, middle and apical), by using bi and tridimensional analyses. The filling data were submitted to ANOVA and Tukey statistical tests, and porosity data to Kruskal-Wallis and Dunn tests, at a 5% significance level. **Result:** S26 and ZOE presented higher filling ability than MTA ($p<0.05$). S26 showed the highest total porosity (number and percentage) ($p<0.05$). In all thirds after 2D and 3D analyses, porosity was higher for S26 in comparison to MTA and ZOE ($p<0.05$). **Conclusion:** Although Sealer 26 presented more porosity, the material was associated with a great filling ability. Micro-CT analysis showed no correlation between filling ability and porosity.

Descriptors: X-Ray Microtomography; porosity; dental materials; endodontics.

INTRODUCTION

Microcomputed tomography (micro-CT) is a non-destructive tool used for different studies in Endodontics as analysis of root canal filling¹, filling ability of the reparative materials² and the evaluation of interface material/dentin associated with different

root canal filling techniques and materials³. Micro-CT is also used for evaluating the volumetric change in endodontic materials, with possible correlation with solubility⁴ and dimensional change after immersion in distilled water².

The porosity of a material may be affected by physicochemical properties. Cements with absence of macropores and lower porosity may present less penetration of oral fluids, bacteria and bacterial toxins into root canal⁵. Pores in sealers appear to be originated from the air trapped in the mass of cement during handling manipulation⁶. Porosity may be analyzed by means of microscopy, using porosimetry by mercury intrusion⁷, or by using microcomputed tomography^{8,9}. De Souza et al.¹⁰, using a 3D model, quantitatively evaluated the degree of porosity of calcium-silicate based materials analyzing the images obtained by microcomputed tomography. 3D models were evaluated and the porosity parameters of each material were obtained by comparison with standard porosity values of Biodentine®. This tool allows a tridimensional mensuration (with volumetric results – mm³) of failures. Surface area, volumetric analysis, the amount of pores and specific characteristics of these structures can be assessed.

Clinically, these physical properties of root-end filling materials are relevant. High porosity values for endodontic materials may affect its physical properties⁷, besides increasing the leakage⁵. A better sealing can be obtained using a material presenting low disintegration and solubility¹¹ and it may be directly related to leakage¹². Also, proper sealing may be related to complete filling of the cavity. Analyzing physicochemical (i.e solubility, pH, filling ability) and biological (i.e biocompatibility, bioactivity) characteristics to the root-end filling material may improve the success of the treatment^{2,4,7,12}.

Mineral Trioxide Aggregate (MTA) is a biocompatible calcium silicate-based material with capacity to induce repair by mineralized tissue¹³. However, the fluid consistency makes it difficult to insert MTA into the root-end cavity, and can harm filling and sealing¹⁴. Sealer 26 (S26) is a resin-based endodontic sealer, composed of bismuth oxide, calcium hydroxide and epoxy resin, and requires higher powder/resin proportion to favor insertion into root-end cavities¹³. It has excellent sealing properties when used as root-end filling material¹⁵, and presents interface adaptation similar to calcium silicate-based cements¹⁶. Zinc oxide and Eugenol cement (ZOE) may also be used with higher powder/liquid proportion¹⁷, favoring its insertion into root-end cavities.

The aim of this study was to evaluate the filling ability and porosity of root-end filling materials by using microcomputed tomography. The null hypothesis was that there is no difference between the materials, and the properties of porosity and filling ability are associated.

MATERIAL AND METHOD

Sample Preparation

Bovine teeth were used in this study. The coronal portion was removed. The root portion was sectioned into 5mm slices, using an Isomet 1000 (Buehler Ltd., Lake Bluff, IL, USA) machine. On dentine surface of each slice, root-end cavities with standardized dimensions (1.5 mm diameter and 3 mm deep) were prepared by making intermittent forward-backward movements¹⁸ using a high-speed 2137 bur (KG SORENSON, Cotia, SP, Brazil). Ultrasonic tip CVD No 6.1107-6 (CVD-Vale, São José dos Campos/SP, Brazil) coupled to an ultrasonic device CVDentus (CVD-Vale, São José

dos Campos/SP, Brazil) was used on the dentinal walls. In order to maintain the same position during the scans, the samples were fixed in culture plates with silicone. Each well of the culture plate was filled with a standard amount of silicone and the samples were placed into this material, at all stages.

Sample Scanning

After cavity preparation, samples with empty cavities were subjected to microtomographic scanings (Micro-CT SkyScan 1176, Bruker micro-CT Kontich, Belgium). The scanning procedure was performed with 50 kV X-ray tube voltages and 800 µA anode current; aluminum filter of 0.5; isotropic voxel of 9 µm; and an evolution cycle of 360°. To standardize the position of samples, specimens were fixed in culture plates with silicone, as already described.

Filling Ability

The prepared cavities were then randomly divided into three groups, according to the material. MTA Angelus (Angelus, Londrina, PR, Brazil) was manipulated by a same operator previously calibrated using powder/liquid proportion of 1g/330µL. S26 (Dentsply, Petropolis, RJ, Brazil) was prepared in a thicker consistency than used as endodontic sealer, in a 4:1 powder/resin proportion (14). ZOE cement (S.S.White Art. Dent. Ltda., Rio de Janeiro, RJ, Brazil) was used in the proportion of 1 g zinc oxide to 0.2 mL eugenol. The cavities were filled with the materials by using a condenser (Trinity, São Paulo, SP, Brazil). Samples were maintained at 37°C and 100% humidity for three times the material setting time. After this period, each filled sample was subjected to another microtomographic scanning, as previously described.

Filling Ability Analysis

The scanned images were reconstructed by using NRecon software (V1.6.4.7; SkyScan, Belgium). The correction parameters smoothing, beam hardening and ring artefacts were carefully adjusted and maintained for all the periods, using values of 7, 20% and 10, respectively. These image parameters were used for all materials, to standardize the analysis process (scanning, reconstruction and analyzing criteria). After reconstruction, images were analyzed by using CTAn software (V1.11.8; SkyScan, Belgium). To obtain the total volume of material in mm³, the area of interest the region of interest (ROI) was defined by previous tests and a calibrated operator made all the analyzes of each sample, excluding the dentin. The binary value (threshold) was adjusted to assure the analysis of the material and the total volume of the material in mm³ from quantitative analysis was obtained. Filling was determined by calculating the subtracting the initial (empty cavities) from the final (filled cavities) assessed volume of each sample. The results were then converted into percentages based on the initial volume. 3D models from each specimen were carefully obtained by using CTVol software (V2.0, SkyScan, Belgium) to analyze qualitatively the data.

Porosity Analysis

Micro-CT proved to be a powerful nondestructive 3D analysis tool for visualizing the porous internal microstructure³. So, at this stage, the filled cavities were analyzed by means of CTAn software

(V1.11.8; SkyScan, Belgium). The porosity parameters for each sample were measured and number and percentage of closed pores were obtained. Total porosity throughout the entire material extent was determined. The limits of each sample were defined by using the “top” and “bottom” tool (Figure 1A). A circular VOI, measuring average 0.75 x 0.75, was determined to each analyzes to exclude any possible artefact related to the sample border. Thus, approximately 1 mm² of margin was excluded. The internal portion of the materials was analyzed by the difference in radiopacity. After this, using the software’s porosity tool, the bidimensional total porosity values were obtained, according to number and percentage of closed pores. This feature allows porosity analysis for individual objects in 2D (bidimensional). It has been defined as the area of any spaces fully surrounded by solid, as a percent of the area of solid plus closed pores. Subsequently, by using the 3D (tridimensional) analysis tool, the number and percentage of closed pores were obtained by means of tridimensional data, providing volumetric data. In this case, a closed pore in 3D is a connected assemblage of space voxels that is fully surrounded on all sides in 3D by solid voxels. In addition a tridimensional model of the samples was obtained by using the CTVol software (V2.0, SkyScan, Belgium).

To obtain more precise and specific analysis, sections with smaller thickness were also analyzed. The “top” and “bottom” tool

was used to define 0.5mm sections to evaluate the porosity in the different cavity thirds: coronal, middle and apical (Figure 1B). The analysis was performed as previously described. For each third, the number and percentage of closed pores were calculated, and 3D models were obtained.

Data from filling analysis were submitted to ANOVA and the Tukey tests, with 5% significance. Porosity data were submitted to the Kruskal-Wallis test, complemented by the Dunn test, with a level of significance of 5%.

RESULT

Filling Ability

Data obtained for filling volumetric analysis are represented in Table 1. Similar filling ability was observed for S26 and ZOE (p>0.05), and both presented higher values than MTA (p<0.05). 3D model analyses (Figure 2) show similar characteristics for S26 and ZOE, in comparison with MTA.

Porosity

The data obtained for porosity are described in Table 2. 2D and 3D analyses presented similar patterns of comparison among the materials. S26 showed higher values for number and percentage of closed pores considering the total extent or each third analysis (p<0.05). MTA and ZOE cement were similar for all the analyses (p>0.05). Considering absolute values for both tests, the bidimensional analysis showed higher values in percentage and lower values for number of pores, while the tridimensional analysis revealed higher number of pores and a lower percentage. 3D models porosity models for each third were obtained (Figure 3). The higher porosity observed to S26 was clearly observed in the qualitative analysis in comparison with MTA and ZOE, in all thirds evaluated.

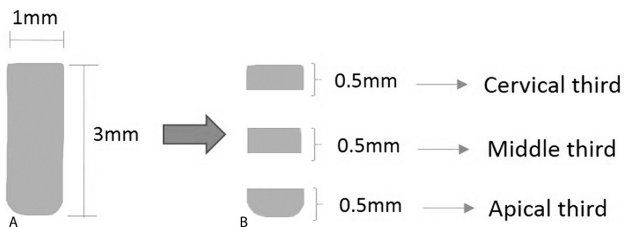


Figure 1. Models used for porosity analysis. (A) Sample analysis for full extent (total porosity); (B) sample analysis for sections (porosity in thirds).

Table 1. Mean percentage values (± standard deviation) of cavity fillings by different root-end filling materials

	MTA	Sealer 26	ZOE
Mean filling	75.66 (8.854) ^b	84.46 (1.959) ^a	84.07 (5.220) ^a

Different letters on the same line indicate statistically significant difference (p<0.05).

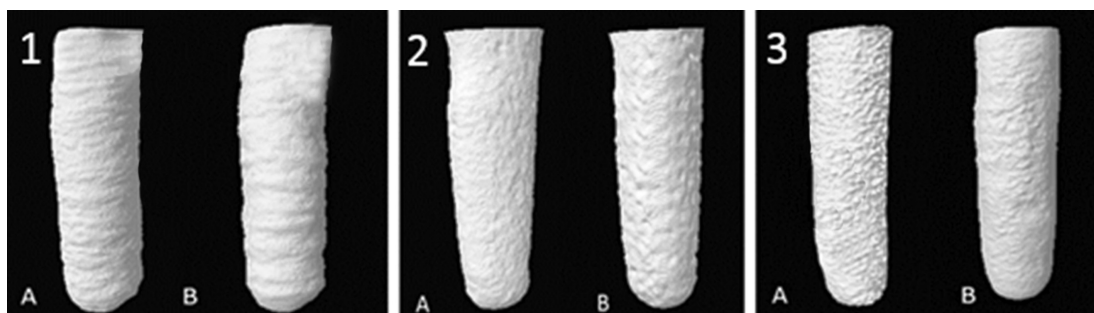


Figure 2. Representative micro-CT tree-dimensional reconstructions of the evaluated samples (1 - MTA; 2 - Sealer 26; 3 - Zinc oxide and eugenol cement). Empty cavity (A) and the cavity filled with each material (B).

Table 2. Porosity values (total number and percentage of closed pores) in cavities filled with different root- end filling materials in 2D and 3D analysis

Materials		MTA	Sealer 26	ZOE	
Total porosity					
No. of closed pores	2D	0.5 ^a	3.12 ^b	0.5 ^a	
	3D	2.62 ^a	12.89 ^b	4.37 ^a	
No. of closed pores	2D	1.24 ^a	5.08 ^b	0.67 ^a	
	3D	0.04 ^a	2.49 ^b	0.19 ^a	
Porosity per thirds					
Cervical Third	No. of closed pores	2D	0.25 ^a	5.62 ^b	0.5 ^a
		3D	2.0 ^a	22.13 ^b	2.5 ^a
	No. of closed pores	2D	0.88 ^a	6.71 ^b	0.63 ^a
		3D	0.24 ^a	1.46 ^b	0.94 ^a
Middle third	No. of closed pores	2D	0.0 ^a	4.5 ^b	0.12 ^a
		3D	0.0 ^a	27.38 ^b	0.12 ^a
	No. of closed pores	2D	0.0 ^a	4.59 ^b	0.31 ^a
		3D	0.0 ^a	2.2 ^b	0.02 ^a
Apical third	No. of closed pores	2D	0.0 ^a	3.37 ^b	0.0 ^a
		3D	0.0 ^a	18.75 ^b	0.0 ^a
	No. of closed pores	2D	0.0 ^a	3.43 ^b	0.0 ^a
		3D	0.0 ^a	1.77 ^b	0.0 ^a

Different letters on the same line indicate statistically significant difference (p<0.05).

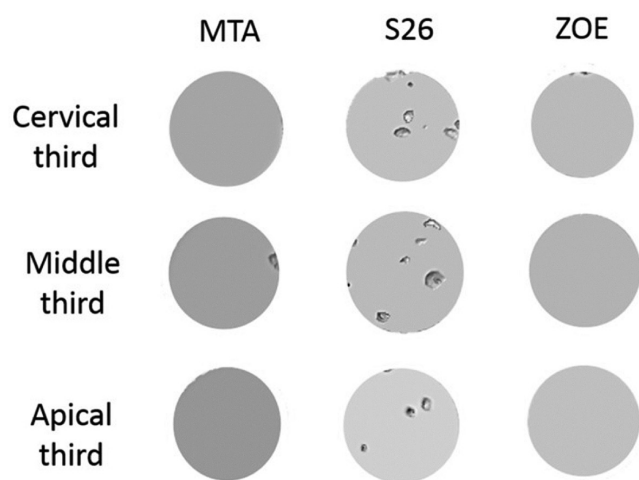


Figure 3. Representative aspect of material porosities observed in the different evaluated thirds for each evaluated material.

DISCUSSION

Micro-CT volumetric analysis shows that S26 and ZOE cement presented greater filling ability than MTA. The larger powder to resin/liquid used to manipulate S26 and ZOE cement made it possible to increase their consistency, favoring their insertion into the root-end cavity. By using microcomputed tomography, Cavenago et al.⁴ evaluated the solubility of MTA with different powder-liquid ratio (4:1, 3:1 and 2:1) before and after immersion in water for 7 days. They observed that the material manipulated with larger quantity of water promoted greater change in volume. Therefore, the difficulty with inserting MTA into the cavity¹³ cannot be improved by changing the powder/liquid proportion.

For this reason, a better fill can be achieved using materials which allow changes in handling, facilitating insertion into the cavity (i.e. S26 and ZOE cement).

Proper cavity filling with a material presenting low disintegration and solubility may allow a better sealing. Favorable sealing ability for some materials may be related to its dimensional stability and filling, which led to less leakage¹¹. Chittoni et al.¹² observed lower bacterial leakage for Sealer 26 in comparison with MTA. Sealer 26 has also demonstrated to prevent bacterial leakage when compared with IRM¹⁵. Amoroso-Silva et al.¹⁶ demonstrated that Sealer 26, MTA and calcium silicate cements were similar after analyzing sealing by fluid leakage and dentinal adaptation. In the present study, similar filling ability was observed for S26 and ZOE, and both presented higher values than MTA. Torres et al.² observed that ZOE presented better filling ability than MTA. Dias et al.¹⁹ observed that the modification of a composite resin with small amounts of zinc oxide (ZnO) microparticles significantly inhibited the *S. mutans* growth on resin surface without significant alterations of its mechanical strength. This filling capacity may be related to the better consistency. The results obtained may suggest this relationship. S26 and ZOE cement presented similar characteristics of filling compared to MTA. The presence of space at the interface of the filling material and the root canal wall can result from deficient adaptation of the filling material to the root dentin²⁰. Despite the excellent properties of MTA, the condensation technique may have some influence in its sealing ability.

Porosity analysis aims to measure the “failure fraction” and empty spaces, counting the spaces and characterizing their connections. The closed pores represent empty spaces completely surrounded by material, which is difficult to be analyzed by conventional methodologies. The use of microcomputed tomography allows

evaluation of these faults¹⁰. Microtomography has been used to evaluate the porosity of different materials^{8,9}. Kerckhofs et al.⁸ aiming to validate micro-CT as an imaging tool for analysis of pores, pointed out errors inherent to the analyses that may result in an incorrect interpretation. Artifacts might influence the quality of the images, challenging image analyses, once the images are reconstructed with several independent detector measurements²¹. As an example, streaking artefacts, which happen generally due to an inconsistency in a single measurement; ring artefacts, which appear due to errors in an individual detector calibration, distortion artefacts, due to the geometry of image reconstruction. In order to minimize these errors, all reconstructions were performed using the artifact reduction tools. De Souza et al.¹⁰ proposed a standard to obtain the threshold, using known values for the porosity of each material. Therefore, the use of microcomputed tomography for porosity analysis also presents variations that must suggest patterns based on the experiment performed and type of sample.

According to the manufacturer (Bruker-microCT, Kontich, Belgium), the CTAn software allows the analysis of porosity of any type of material. In this study, samples were scanned at a high resolution (9µm), considered proper for quantifying pores²². During the reconstruction of images, standardized parameters were used in order to decrease artefacts. Analysis of the total sample extent presented more difficulty with defining the parameters, especially the histogram. Therefore, analysis of smaller sections (or thirds) is considered important for correct analysis.

As regards the results of this study, the two measurement analyses showed greater porosity for S26 in comparison with MTA and ZOE cement, for both number and percentage of pores. However, the 2D analyses showed higher percentage values, while the 3D presented a higher number of pores. In microscopic 2D images, a

failure may appear as a closed pore, while in the 3D evaluation this pore is considered connected to the external space, which is one advantage of 3D analysis in comparison with conventional methods. Therefore, 3D analysis is indicated for this type of porosity analysis. Bidimensional analysis was evaluated to obtain complementary data. In the present study, bidimensional and tridimensional analysis presented correlated results for material porosities.

The analysis of porosity did not show correlation with filling data, since S26 and ZOE cement promoted greater filling. Otherwise, more porosity was observed for S26 when compared with MTA and ZOE cement, rejecting the null hypothesis. The immediate porosity (after the material set) is related to the material composition and hydration mechanism, especially to water/powder proportion for MTA²³. MTA has demonstrated porosity in different formulations, especially after long evaluation periods³. The low porosity observed for MTA in the present study may be related to the different methodologies and analysis performed immediately after cavity filling. Mutal, Gani⁶, after a qualitative analysis of pores in endodontic sealers, including zinc oxide and eugenol cements and Sealer 26, observed that the frequency and size of pores were related to the consistency of the material, and was increased for sealers with calcium hydroxide, such as Sealer 26.

CONCLUSION

Based on the methodology used and the results obtained, we could conclude that Sealer 26 and ZOE cement showed better filling ability in comparison to MTA. On the other hand, Sealer 26 presented porosity higher in number and percentage than MTA and zinc oxide and eugenol.

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CONFLICTS OF INTERESTS

The authors declare no conflicts of interest.

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