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Effect of Different Dimensions of Test Samples on the Volumetric Change Assessment Of Endodontic Materials

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New methodologies using micro-CT to evaluate solubility besides dimensional and morphological changes of endodontic materials are proposed. However, there is no standardization in the methods. The aim of this study was to assess the effect of different dimensions of test samples on volumetric change evaluation of different endodontic materials. AH Plus, FillCanal and Sealapex root canal sealers, Biodentine, IRM and MTA root-end filling cements were used in the tests. Samples of each material with a thickness of 1.5 mm and different diameters were manufactured: 6.3, 7.75, and 9.0 mm. The samples were scanned in micro-computed tomography (micro-CT) after setting and after 7 days of immersion in distilled water. The volumetric change was evaluated by means of the difference in the total volume of the specimens before and after immersion. Data were submitted to ANOVA and Tukey tests ($p<0.05$). The size of the samples did not affect the percentage of volumetric change of the materials ($p>0.05$). All sample sizes had greater volume loss for Sealapex among the sealers and Biodentine for the cements ($p<0.05$). In conclusion, Biodentine and Sealapex had the highest volume loss after immersion. Samples with 1.5 mm thickness, and diameters ranging between 6.3 and 9.0 mm can be used to assess the stability of endodontic materials using micro-CT without affecting the percentage of volumetric change.

Key Words: endodontics, physical properties, root canal obturation, x-ray microtomography.

Introduction

Root canal sealers associated to gutta-percha cones are used for a three-dimensional filling of the root canals, preventing leakage and promoting repair of periapical tissues (1). Endodontic materials are also indicated for root-end filling, pulp capping, root perforation treatment, and regeneration (2).

An ideal endodontic material should be insoluble in tissue fluids, and dimensionally stable (3), since solubility and dimensional changes might compromise the sealing ability leading to reinfection (4). Therefore, research on different cements currently available is performed to select materials based on their stability (5).

Many differences in the solubility test may be observed and the discrepancy of results could be related to their methodology (6). Solubility of a sealer may not be related to its dimensional stability and sealing ability (3). Moreover, solubility of a sealer can be compensated by its fluid absorption (7).

Micro–computed tomographic (micro-CT) is a high-resolution technology that allows a nondestructive imaging analysis (8). Micro-CT is able to develop accurate three-dimensional models with repeated exposures and acquisition of data (8). The use of micro-CT has increased in dentistry research (9). Endodontic micro-CT analysis allows the evaluation of the solubility besides dimensional and morphological changes of cements and sealers comparing their volumetric change after immersion in a fluid (7, 10, 11).

Although the volumetric change assessment using micro-CT is a methodology widely used in current research, there is a lack of standardization in the method. The absence of defined protocols for studies using micro-CT may influence the results (12), compromising the comparison among different investigations. Therefore, this gap in the literature leads us to investigate the influence of the amount of material exposed to the fluid on solubilization and dimensional change.

Therefore, the aim of this study was to evaluate the effect of different sample sizes on the volumetric change evaluation of endodontic materials based on different compositions to determine whether their use can influence endodontic research outcomes. The null hypothesis was that there would be no difference on the volumetric change of the materials using different dimensions of test samples.

Material and Methods

Specimen Preparation

Based on a previous study (4), circular plastic molds measuring 1.5 mm thickness and 6.3 mm in diameter, 1.5 mm thickness and 7.75 mm in diameter, and 1.5 mm thickness and 9.0 mm in diameter were placed on a glass plate covered with cellophane film and filled with the endodontic materials (Table 1). Wet gauze was placed between the specimen and the glass plates for materials that require moisture for setting. (11). A total of 108 samples was manufactured and divided into 6 groups (n = 18 for each material evaluated, and n = 6 for each dimension of test sample). The sample size for this study was calculated based on a previous investigation that evaluated the volumetric change of endodontic materials (13). G*Power 3.1.7 for Windows (Heinrich-Heine-Universitat Dusseldorf, Dusseldorf, Germany) was used for the sample calculation. One-way analysis of variance was used, with alpha type error of 0.05, beta power of .80 and effect size of 0.89. Six specimens per group were calculated as the necessary sample size. The samples were kept at 37 °C and 95% humidity for 2 days for the cements and 7 days for the sealers. After setting, the samples were stored in a desiccator under vacuum for 24 h.

Volumetric Change Assessment

The volumetric change of the materials was evaluated according to a previous study (11). The samples were scanned by micro-CT SkyScan 1176 (Bruker, Kontich, Belgium) at 80 kV voltage, 300 µA current, 18 µm voxel size, copper and aluminum (Cu + Al) filter and 360° rotation. After the first scanning, the materials were placed in closed plastic flasks containing 5.0 mL of distilled and deionized water for the test samples with 6.3 mm in diameter, 7.5 mL for 7.75 mm, and 10.0 mL when using the test samples with 9.0 mm (4). The specimens were kept in an oven at 37 °C for 7 days, and their position was inverted in the plastic flasks after 3.5 days to allow that the surface of the materials remain in contact with the liquid for the same period of time (11). After the complete period, the samples were placed in a desiccator under vacuum for 24 h and scanned again. The reconstruction of the images was performed using NRecon software (V1.6.10.4; Bruker, Kontich, Belgium). The correction parameters for smoothing, beam hardening and ring artefacts were individually defined for each material. The same parameters were used for the same material at the different periods. The images obtained before and after immersion were superimposed with geometric alignment using the Data Viewer software (V1.5.2.4; Bruker, Kontich, Belgium). Quantitative analyses were then performed using CTAn software (V1.15.4.0; Bruker-MicroCT, Kontich, Belgium), which allowed the total volume

Table 1. Endodontic materials, their manufacturers, composition, and proportion used

Material	Manufacturer	Composition	Ratio
AH Plus	DentsplyDeTrey, Konstanz, Germany	Paste A: bisphenol epoxy resin–A, bisphenol epoxy resin–F, calcium tungstate, zirconium oxide, silica, iron oxide pigments. Paste B: dibenzyldiamine, aminodiamantana, tricyclodecane–diamine, calcium tungstate, zirconium oxide, silica, silicone oil.	1 g : 1 g
Fill Canal	Technew Com. Ind. Ltda. Rio de Janeiro, RJ, Brazil	Powder: hydrogen resin, bismuth subcarbonate, barium sulfate and sodium borate Liquid: eugenol and sweet almond oil.	1 g : 0.2 mL
Sealapex	SybronEndo – Sybron Dental Specialties, Glendona, CA, USA	Base paste: sulphonamide resin, N-ethyl toluene, silicon dioxide, zinc oxide, calcium oxide; Catalyst paste: isobutyl salicylate resin, silicon dioxide, bismuth trioxide, titanium dioxide, pigments	1 g : 1 g
Biodentine	Septodont; Saint-Maur-des-Fossés, France	Powder: tricalcium silicate, calcium carbonate, zirconium oxide, dicalcium silicate, calcium oxide, iron oxide Liquid: aqueous solution of a hydrosoluble polymer with calcium chloride	1 g : 6 drops
IRM	Dentsply, Caulk Milford,DE	Powder: zinc oxide, poly methyl methacrylate Liquid: eugenol, acetic acid	1 g : 0.2 mL
MTA	Angelus, Londrina, PR, Brazil	Powder: tricalcium silicate, dicalcium silicate, tricalcium aluminate, calcium oxide, bismuth oxide Liquid: distilled water	1 g : 0.33 mL

of material to be calculated in mm³. The volumetric change between the baseline and the experimental period was calculated.

Statistical Analysis

The results obtained were submitted to a normality test, and then to the parametric ANOVA statistical test and the Tukey multiple comparison test, with 5% significance level.

Results

The results regarding the evaluation of the root canal sealers are showed in Table 2 and illustrated in Figure 1. All sealers maintained their percentage of volumetric change, regardless of the diameter of the specimen ($p>0.05$). AH Plus presented volume gain, while Fill Canal and Sealapex presented a volumetric reduction. Sealapex had greater volumetric change than the other materials ($p<0.05$).

When evaluating the root-end filling materials, all the cements presented loss of volume. In a similar way to the root canal sealers, the materials kept their percentage of volumetric change in all specimen sizes ($p>0.05$). Biodentine presented the greatest volume loss, followed by MTA and IRM ($p<0.05$). The results are presented in Table 3 and illustrated in Figure 1.

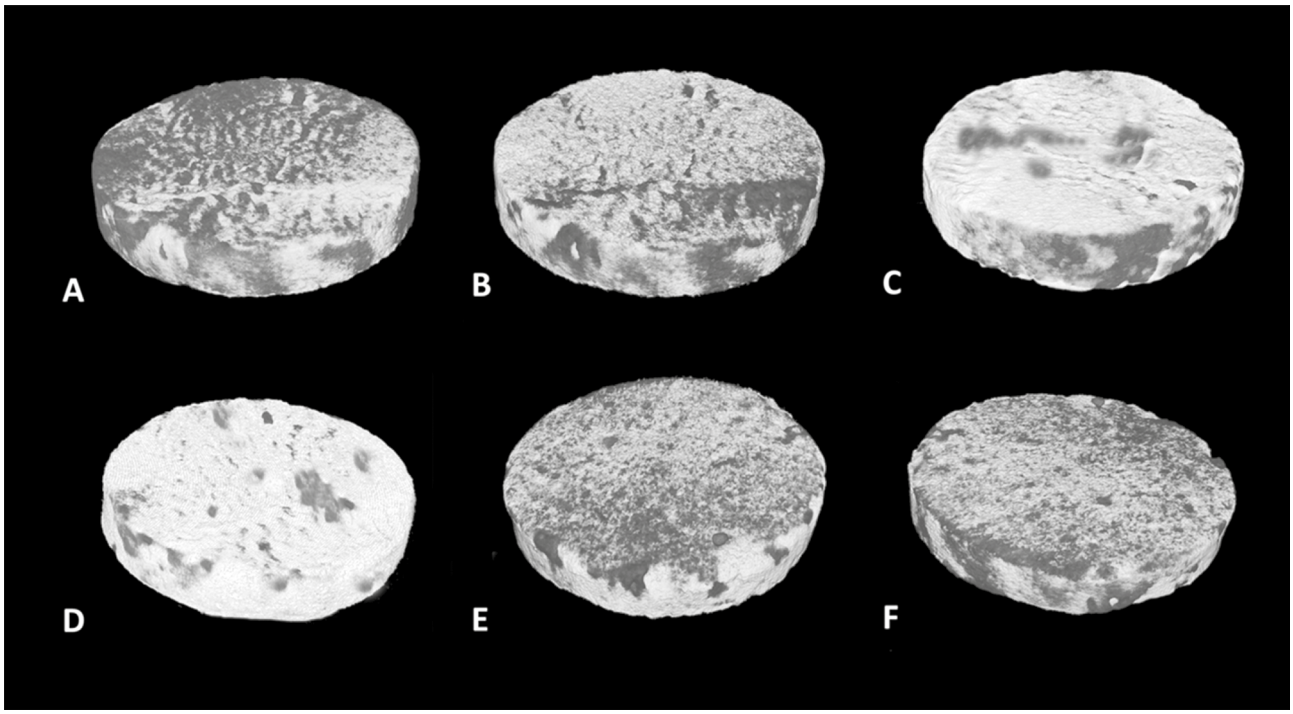

Figure 1 - 3D models representing the volumetric change assessment before (white) and after (grey) immersion of the samples in distilled water for AH Plus (A), Fill Canal (B), Sealapex (C), Biodentine (D), IRM (E), and MTA (F). Models with highlighted white color indicate greater volume loss.

Table 2. Volumetric change values (%) using different dimensions of test samples (mean and standard deviation) observed in root canal sealers

Volumetric change (%)	AH Plus	Fill Canal	Sealapex
6,30 x 1,5 mm	1.10 (0.16)A,c	-0.90 (0.41)A,b	-1.69 (0.68)A,a
7,75 x 1,5 mm	0.90 (0.05)A,c	-0.90 (0.17)A,b	-1.51 (0.21)A,a
9,00 x 1,5 mm	0.98 (0.20)A,c	-1.18 (0.24)A,b	-1.76 (0.40)A,a

The same capital letter in the same column indicate no statistically significant difference between the dimensions of test samples ($p>0.05$). Different lower case letters on the same row indicate statistically significant difference between the sealers ($p<0.05$). Negative values indicate volume loss

Table 3. Volumetric change values (%) using different dimensions of test samples (mean and standard deviation) observed in endodontic cements

Volumetric Change (%)	Biodentine	IRM	MTA
6,30 x 1,5 mm	-5.20 (0.71)A,a	-0.59 (0.13)A,c	-1.08 (0.04)A,b
7,75 x 1,5 mm	-4.79 (0.85)A,a	-0.59 (0.09)A,c	-0.99 (0.05)A,b
9,00 x 1,5 mm	-5.58 (0.53)A,a	-0.47 (0.18)A,c	-1.07 (0.33)A,b

The same capital letter in the same column indicate no statistically significant difference between the dimensions of test samples ($p>0.05$). Different lower case letters on the same row indicate statistically significant difference between the cements ($p<0.05$). Negative values indicate volume loss.

Discussion

Physicochemical properties such as solubility and dimensional stability of the endodontic materials may be related to their sealing ability (4). Thus, materials with low solubility and dimensional stability may decrease the possibility of gap formation between root canal dentine and the filling material (7).

According to the ISO 6876 (14) or ANSI/ADA (15) specifications, solubility evaluation is based on differences in material weight before and after water immersion and dimensional stability is measured in a linear direction. However, these tests can present some limitation once materials can present fluid caption, and the shrinkage or expansion can occur for all directions (16). Based on these limitations, micro-CT has been proposed as an alternative method to evaluate in 3D the physical changes after immersion in a fluid (7,10,11,17). Thus, based on volume changes the stability of the materials can be evaluated (7).

Although several studies have been performed using micro-CT to evaluate the volumetric change of endodontic cements and sealers, it is possible to observe an absence of standardization of the methodologies. The lack of a defined protocol for acquiring and analysing micro-CT images may compromise the scientific impact of the studies (12). This gap on the literature led to require researches to investigate the test variables to assess their influence on the results of endodontic studies (18).

Carvalho-Junior et al. (4) proposed smaller dimensions for samples used in solubility and dimensional change tests. The authors observed a correlation among results of the different dimensions, showing that is possible to decrease the amount of materials without affecting the accuracy of the tested methods. The current article used the sample sizes based on this previous study and our results are in agreement, since a variation in the diameter of the samples does not interfere with the volumetric change, accepting our null hypothesis.

The same scanning parameters were used for all materials in order to standardize the test, even with endodontic materials with different radiopacities (11) since during the reconstruction of the images it is possible to reduce artifacts without affecting the objective analysis of the image (9). In addition, the images were acquired with a proper resolution (18 µm) and the parameters for artifact correction were defined for each material, in order to reduce the amount of noise, enhancing the contrast (19). Furthermore, the segmentation of the material from the background is substantial for an accurate analysis, avoiding under or overestimation of the real volume (19). Thus, our analysis was performed using objective segmentation, by means of an "automatic threshold". This method is reliable and should be preferable in micro-CT studies due to the absence of human influence (19).

The current study showed that AH Plus presented the greatest volumetric stability. These results are in agreement with previous studies showing low volumetric changes for this sealer (7,10,11). Moreover, AH Plus was the only sealer that showed a volume increase. The low solubility added to its dimensional expansion (10) could explain these findings. The strong cross-links in epoxy resin-based sealers justify the volumetric stability of AH Plus (20).

On the other hand, Biodentine and Sealapex had the greatest volumetric reduction after immersion in distilled water. Previous studies showed that Biodentine has greater solubility than the recommended by the ISO/ADA standards (21,22). The presence of polycarboxylate in its composition presents a surfactant effect and may disperse the cement particles (21). The volume loss of Biodentine can also be associated with high amounts of ions release, an important factor for its bioactivity (22). The high volume loss in Sealapex can occur also due its solubility (16), which is related to its non-homogeneous setting reaction, resulting in a fragile matrix (16, 20).

Fill Canal and IRM are zinc oxide and eugenol-based materials. The volumetric loss of these materials may occur due to the loss of eugenol by a leaching effect (10). However, the present study showed that the volumetric reduction was low, probably because eugenol is not water-miscible (17). Although with lower values than Biodentine, MTA also had a decrease in volume after immersion (17). The solubility of MTA was described with a decreasing rate, and its soluble fraction is mainly composed by calcium hydroxide, which is capable of maintaining a high pH in aqueous solution (23).

It is important to note that the contact area in the current study between the materials and water was higher than in a clinical situation, which may imply a higher dissolution and shrinkage (24). Moreover, the methodology used to volumetric change assessment kept the samples in a desiccator for 24 h before the scanning procedures in micro-CT. This procedure was performed in order to avoid underestimation in the solubility of the cements, since the materials may present a volumetric expansion after water immersion, when no pre-storage desiccation treatment is applied (22). However, the volume reduction could not be entirely due to the solubility, but as well as by

evaporation of the water during the drying of the samples (25).

In conclusion, within the limitations of this *in vitro* study, samples with 1.5 mm thickness, and diameters ranging between 6.3 and 9.0 mm can be used to assess the stability of endodontic materials using micro-CT without affecting the percentage of volumetric change.

Resumo

Novas metodologias utilizando micro-CT são propostas para avaliar a solubilidade além de alterações dimensionais e morfológicas em materiais endodônticos. No entanto, não há padronização nos métodos. O objetivo deste estudo foi avaliar o efeito de diferentes dimensões de corpos de prova na avaliação da alteração volumétrica de diferentes materiais endodônticos. Os cimentos obturadores AH Plus, FillCanal e Sealapex e os cimentos retrobturadores Biodentine, IRM e MTA foram utilizados nos testes. Foram confeccionadas amostras de cada material com espessura de 1.5 mm e diâmetros diferentes: 6.3, 7.75 e 9.0 mm. As amostras foram escaneadas em microtomografia computadorizada (micro-CT) após a presa e após 7 dias de imersão em água destilada. A alteração volumétrica foi avaliada por meio da diferença no volume total dos corpos de prova antes e após a imersão. Os dados foram submetidos aos testes ANOVA e Tukey ($p<0,05$). A dimensão das amostras não afetou o percentual de alteração volumétrica dos materiais ($p>0,05$). Todos os diâmetros de amostra mostraram maior perda de volume para Sealapex entre os cimentos obturadores e Biodentine entre os cimentos retrobturadores ($p<0,05$). Como conclusão, Biodentine e Sealapex mostraram a maior perda volumétrica após a imersão. Amostras com 1.5 mm de espessura e diâmetros variando entre 6.3 e 9.0 mm podem ser usadas para avaliação da estabilidade de materiais endodônticos utilizando micro-CT, sem influenciar no percentual de alteração volumétrica.

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