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Research Article

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A micro-computed tomographic study using a novel test model to assess the filling ability and volumetric changes of bioceramic root repair materials

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Conflict of Interest
No potential conflict of interest relevant to this article was reported.

ABSTRACT

Objectives: New premixed bioceramic root repair materials require moisture for setting. Using micro-computed tomography (micro-CT), this study evaluated the filling ability and volumetric changes of calcium silicate-based repair materials (mineral trioxide aggregate repair high-plasticity [MTA HP] and Bio-C Repair, Angelus), in comparison with a zinc oxide and eugenol-based material (intermediate restorative material [IRM]; Dentsply DeTrey).
Materials and Methods: Gypsum models with cavities 3 mm deep and 1 mm in diameter were manufactured and scanned using micro-CT (SkyScan 1272. Bruker). The cavities were filled with the cements and scanned again to evaluate their filling capacity. Another scan was performed after immersing the samples in distilled water for 7 days to assess the volumetric changes of the cements. The statistical significance of differences in the data was evaluated using analysis of variance and the Tukey test with a 5% significance level.
Results: Bio-C Repair had a greater filling ability than MTA HP ($p < 0.05$). IRM was similar to Bio-C and MTA HP ($p > 0.05$). MTA HP presented the largest volumetric change ($p < 0.05$), showing more volume loss than Bio-C and IRM, which were similar ($p > 0.05$).
Conclusions: Bio-C Repair is a new endodontic material with excellent filling capacity and low volumetric change. The gypsum model proposed for evaluating filling ability and volumetric changes by micro-CT had appropriate and reproducible results. This model may enhance the physicochemical evaluation of premixed bioceramic materials, which need moisture for setting.

Keywords: Biocompatible materials; Endodontics; Methods; X-ray microtomography

INTRODUCTION

Mineral trioxide aggregate (MTA) is a material indicated for treatments of the vital pulp, regenerative endodontic therapies, apical barriers, perforation repairs, and root-end filling due to its biocompatibility, bioactivity, and proper sealing [1,2]. Therefore, MTA represents the gold standard for comparisons with new materials [3]. However, MTA presents some disadvantages, such as a long setting time, difficulties in handling, and a risk of tooth discoloration [1,3]. Since a number of bioactive endodontic cements based on calcium

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silicates have been introduced to the market aiming to overcome the drawbacks of MTA, further investigations using accurate methodologies should be performed in order to confirm their long-term effectiveness [1].

MTA repair high-plasticity (MTA HP; Angelus, Londrina, PR, Brazil) has an organic plasticizer added to the distilled water in order to improve its physicochemical properties, such as plasticity [4]. Moreover, in MTA HP, bismuth oxide was replaced by calcium tungstate as a radiopacifier to prevent tooth staining [4]. Previous studies observed appropriate biological and physicochemical properties of this material [4-7]. Bio-C Repair (Angelus) is a new, premixed, ready-to-use bioceramic repair material [8]. Bio-C Repair showed proper biological properties, promoting high cell viability, cell adhesion, and cell migration, as well as inducing biomineralization [8,9]. However, no study has evaluated the physicochemical properties of Bio-C Repair.

From a clinical point of view, a proper filling is directly correlated to the prevention of bacterial leakage [10]. Thus, a mineral precipitate at the interface between the calcium silicate-based cement and dentin improve the sealing ability of this class of materials [11]. Aiming to assess the filling ability of endodontic materials, micro-computed tomography (micro-CT) has been used as a tool that enables reproducible analysis, promoting accurate nondestructive volumetric measurements of filling materials [10,12]. Furthermore, micro-CT has been used to assess the dimensional stability and solubility of endodontic materials based on calcium silicate by calculating the volumetric change after immersion of these materials in a fluid environment [13,14]. However, no studies have yet evaluated the filling ability and volumetric changes of MTA HP and Bio-C Repair.

Calcium silicate-based materials need water to set through a hydration reaction [3]. Calcium sulfate, usually in the form of dehydrate (gypsum), can be used to control the rate of the setting process [15]. Therefore, plaster molds are recommended for the evaluation of these materials, which should be kept at 37°C and > 95% humidity for 24 hours before testing [16].

Since micro-CT can enhance the conventional evaluation of reparative materials [12], in light of the importance of assessing the physical properties of newly developed premixed bioceramic materials, a method that takes into account the fact that calcium silicate-based cements require moisture for setting should be applied. Therefore, the aim of this study was to use micro-CT to assess the filling capacity and volumetric changes of repair materials (MTA HP and Bio-C Repair), in comparison with a zinc oxide and eugenol-based material (intermediate restorative material [IRM]), using plaster models. The null hypothesis was that there would be no significant difference among these materials for both properties evaluated.

MATERIALS AND METHODS

The specification of the endodontic materials used in this study are described in **Table 1**.

Micro-computed tomography assessment of filling ability and volumetric changes

According to ISO 6876/2012, gypsum molds should be used to assess materials that require moisture for setting [17]. Therefore, metal molds were used to fabricate standardized gypsum-based models (Durone IV Salmon, Dentsply, Petrópolis, RJ, Brazil) with cavities measuring 3

Table 1. The manufacturers, composition, and proportions of the endodontic materials used in this study

Material	Manufacturer	Composition	Proportion
Bio-C Repair	Angelus, Londrina, PR, Brazil	Calcium silicate, calcium aluminate, calcium oxide, zirconium oxide, iron oxide, silicon dioxide, dispersing agent	Ready to use
MTA HP	Angelus, Londrina, PR, Brazil	Powder: tricalcium silicate, dicalcium silicate, tricalcium aluminate, calcium oxide, calcium tungstate Liquid: water and plasticizer	1 g powder: 300 µL liquid
IRM	Dentsply DeTrey, Konstanz, Germany	Powder: zinc oxide, polymethyl methacrylate Liquid: eugenol, acetic acid	1 g powder: 200 µL liquid

MTA HP, mineral trioxide aggregate repair high-plasticity; IRM, intermediate restorative material.

mm deep and 1 mm in diameter (n = 6 per group). The sample size for this study was calculated based on a previous investigation with similar methodology [18]. 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.

Although IRM is a zinc oxide and eugenol-based cement, the setting of all materials was assessed before the micro-CT procedure. The gypsum-based models were scanned using micro-CT (SkyScan 1272, Bruker, Kontich, Belgium). The scanning parameters were: 100 kV X-ray tube voltage and 100 µA anode current; Cu filter of 0.11 mm; isotropic voxel of 15 µm; and an evolution cycle of 180°. After the first scan, the cavities were immersed in distilled water at 37°C for 24 hours. The samples were then filled with each material by a single calibrated operator and stored in an oven at 37ºC and 95% humidity for 24 hours. New scans were performed after setting of the materials and after 7 days of immersion in distilled water. The images were reconstructed using NRecon software (V1.6.10.4, Bruker-MicroCT, Kontich, Belgium). The correction parameters when evaluating the empty models were: 2 for smoothing, 0 for beam hardening, and 7 for ring artifacts. After filling, the values were: 5 for smoothing, 10 for beam hardening and 10 for ring artifacts for IRM; 5 for smoothing, 0 for beam hardening, and 10 for ring artifacts for MTA HP; and 3 for smoothing, 0 for beam hardening, and 10 for ring artifacts for Bio-C Repair. The reconstructed images were superposed by Data Viewer software (v1.5.2.4, Bruker). The total volume (mm^3) of the materials was calculated by CTAn software (v1.15.4.0, Bruker-MicroCT), using global thresholding. The volume of the empty cavities was used to determine the total volume of each cavity for calculating the percentage filled by each material. The difference between the volume of the filled cavities before and after immersion was considered the volumetric change of each cement. A schematic figure illustrating the methodology is shown in **Figure 1**.

Statistical analysis

The results obtained were analyzed using a normality test (Kolmogorov-Smirnov). The statistical analysis was performed with 1-way analysis of variance and the Tukey parametric test (α = 5%).

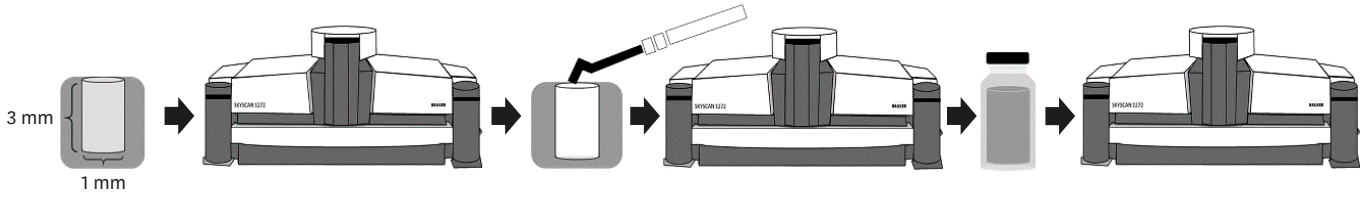

Figure 1. Schematic figure of the assessments of filling ability and volumetric changes. Gypsum–based models with cavities measuring 3 mm deep and 1 mm in diameter were manufactured and scanned using micro-computed tomography before and after filling. The samples were immersed in distilled water for 7 days, and another scan was performed.

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RESULTS

The results are presented in **Table 2**. Bio-C Repair had the highest values for filling ability, showing better results than MTA HP ($p < 0.05$). IRM was similar to Bio-C and MTA HP ($p > 0.05$). Regarding volumetric changes, all materials showed volume loss after immersion in distilled water. MTA HP showed the largest volumetric change ($p < 0.05$), while Bio-C and IRM were similar ($p > 0.05$). Three-dimensional models illustrating the cavities filled with each material are presented in **Figure 2**.

DISCUSSION

In this study, 2 calcium silicate-based cements were compared with a zinc oxide and eugenol-based material regarding their filling ability and volumetric changes through micro-CT imaging of a new gypsum model. Since significantly different results were observed for the materials, our null hypothesis was rejected.

A long setting time has been discussed as a potential drawback of calcium silicate-based materials [19]. Therefore, new hydraulic premixed bioceramic root repair cements have been developed with the goal of improving this disadvantage [19]. This class of materials needs the presence of water to set and harden, and the ISO 6876/2012 standard recommends that a gypsum mold should be used to test materials that require moisture for setting [17]. As a result, new methodologies need to be developed to properly evaluate these new materials. Moreover, calcium silicate-based cements may be kept moist when used clinically to

Table 2. Results for filling ability and volumetric changes of the endodontic materials

Test/material	Bio-C Repair	MTA HP	IRM
Filling ability (%)	97.30 ± 2.01[a]	89.80 ± 4.94[b]	92.30 ± 5.37[a,b]
Volumetric change (%)	−2.36 ± 0.75[b]	−3.67 ± 0.86[a]	−2.10 ± 0.83[b]

The values are mean ± standard deviation. Different lowercase letters on the same line indicate statistically significant differences between the different cements ($p < 0.05$) (1-way analysis of variance and Tukey test). MTA HP, mineral trioxide aggregate repair high-plasticity; IRM, intermediate restorative material.

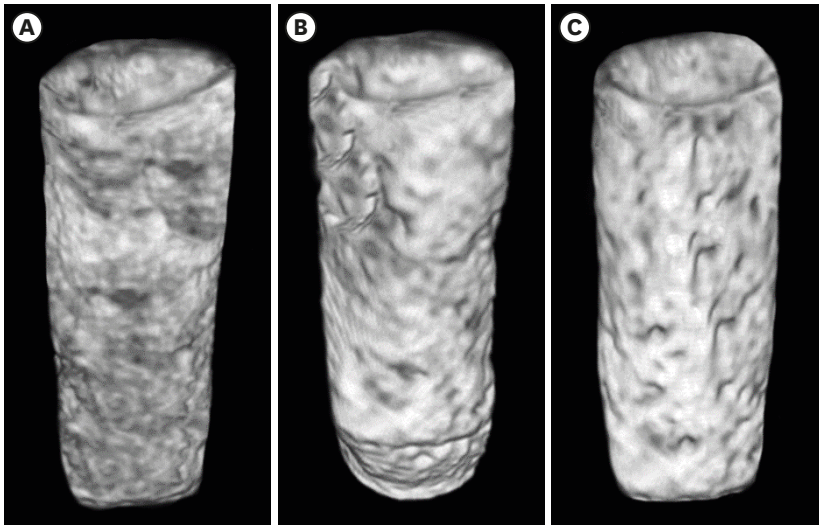

Figure 2. Three-dimensional models illustrating microtomographic images of the filling ability of Bio-C Repair (A), mineral trioxide aggregate repair high-plasticity (B), and intermediate restorative material (C) in CTVox software.

prevent shrinkage and cracking [20]. Based on this important information and considering that conventional tests are not appropriate to evaluate the physicochemical properties of hydraulic materials [21], our study developed a plaster model with standardized cavities to evaluate these cements using micro-CT. Cavities that were 3 mm deep and 1 mm in diameter were manufactured because in surgical endodontics, after apicectomy the apical canals are prepared with these dimensions with ultrasonic tips and subsequently are sealed with a root-end filling material, which is one of the indications of reparative cements [1,22].

In the present study, MTA HP was used since it presented adequate physicochemical and biological properties with improved flow ability when compared to WMTA [4]. IRM is a zinc oxide and eugenol-based cement that was added because it is commonly used as a control for comparisons with calcium silicate cements [20,23]. Our results showed that Bio-C Repair had the highest filling capacity, although its filling capacity was similar to that of IRM. The suitable filling capacity of zinc oxide and eugenol cement has been reported previously, and it showed better results than MTA [18]. This characteristic could be explained by the low porosity and satisfactory interfacial adaptability of IRM [20,23]. In contrast, a comparison between MTA and IRM to seal large furcation perforations using a dye-extraction leakage method showed that ProRoot MTA had better sealing capacity [24]. In addition, ProRoot MTA obtained better results than IRM in relation to bacterial microleakage [25].

Although this is the first study to evaluate the physical properties of Bio-C Repair, our findings can likely be explained as resulting from its suitable consistency and viscosity. Moreover, a previous study evaluating TotalFill BC RRM Putty (FKG, La Chaux-de-Fonds, Switzerland), a ready-to-use repair material, showed an adequate sealing ability and marginal adaptation of apical plugs for this cement [26]. However, even though a plasticizer was added to the distilled water in MTA HP liquid, increasing the flow of this cement in comparison with MTA Angelus [4], this material showed lower percentage of filling than Bio-C. A possible explanation for this result is that flow and filling properties may not be correlated [12,27].

Reparative materials should have low solubility when in contact with the tissue fluid [28]. Although the ISO 6876 standard specifies a procedure for evaluating the solubility of endodontic materials [17], this methodology is not ideal for assessing calcium silicate-based cements [13], since during this test, the mass loss of hydraulic materials occurs not only due to their solubility, but also as a consequence of the evaporation of the liquid contained in these materials during drying of the specimens, which is required in this method [21]. A recent publication stated that another important drawback of the ISO standard for assessing the solubility of endodontic materials is that the materials have a large contact area with the environment [29]. Therefore, the ISO model is not representative of clinical conditions [29]. In contrast, the dimensions of the cavities made in our study could represent clinical situations more realistically, since the materials are kept humid and contact with the fluid only occurs at the material's surface. In this respect, the physical properties of materials should be assessed with more advanced methods [30]. Using micro-CT to assess volumetric changes enables the concomitant evaluation of the properties of solubility and dimensional stability [13]. The possibility of evaluating both of these properties is an advantage of micro-CT, as the absorption of fluids could compensate for solubility when assessing materials that present fluid uptake.

The volumetric change assessment showed that all the materials had a volume loss of around 3%. However, the values were higher for MTA HP than for the other cements, corroborating

a publication that found high solubility values for this cement [31]. In contrast, a previous study evaluating the solubility of IRM showed that it had the lowest solubility among all filling materials evaluated up to 30 days, in agreement with our results [32]. No previous study has assessed the solubility of Bio-C Repair, meaning that there are no reference values for a comparison. Therefore, the data and results presented in the current study could serve as a reference for future investigations of new materials. Moreover, the methodology proposed in our study may complement the evaluation of biological properties of calcium silicate cements. Thus, the proper filling ability and low volumetric change of these new materials, in addition to their cytocompatibility and bioactive potential, may suggest that they are promising for clinical application.

CONCLUSIONS

In conclusion, Bio-C Repair is a new endodontic material with an adequate filling capacity and low volumetric change. The gypsum model proposed for evaluating filling ability and volumetric changes using micro-CT had appropriate and reproducible results. This model may enhance the physicochemical analysis of calcium silicate-based materials, which need moisture to set.

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