UNESP - Universidade Estadual Paulista
"Júlio de Mesquita Filho"
Faculdade de Odontologia de Araraquara

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Desenvolvimento de novas metodologias utilizando Micro-CT para avaliação de propriedades físico-químicas de materiais reparadores e cimentos endodônticos

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Tese apresentada à Universidade Estadual Paulista (Unesp), Faculdade de Odontologia, Araraquara, para obtenção do título de Doutor em Odontologia, na Área de Endodontia

Orientador: Mário Tanomaru Filho

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DADOS CURRICULARES

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“Agir, eis a inteligência verdadeira. Serei o que quiser. Mas tenho que querer o que for. O êxito está em ter êxito, e não em ter condições de êxito. Condições de palácio tem qualquer terra larga, mas onde estará o palácio se não o fizerem ali?”
Fernando Pessoa*

* Fernando Pessoa apud Soares, B. Livro do desassossego. Lisboa: Ática; 1982. v.2.

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RESUMO

Este estudo avaliou novas propostas e parâmetros necessários para análise fisico-química de materiais empregando micro-CT. Os materiais utilizados nos subprojetos 1, 2, 3 e 5 foram: AH Plus, Fill Canal e Sealapex; Biodentine, IRM e MTA. Foram aplicados os testes estatísticos ANOVA/Tukey e teste T (α=.05). **Subprojeto 1-** avaliou por micro-CT o efeito do tempo de imersão em água destilada (7 e 30 dias) na alteração volumétrica de cimentos endodônticos inseridos em cavidades simuladas em moldes de resina. Todos os cimentos obturadores apresentaram diferença na porcentagem de alteração volumétrica de 7 para 30 dias de imersão. Conclui-se que perídos maiores de imersão podem influenciar as alterações volumétricas de materiais. **Subprojeto 2-** avaliou a influência do tamanho de corpos de prova (espessura de 1,50 mm e diâmetros internos de 6,30, 7,75 e 9,00 mm) na alteração volumétrica de materiais após imersão em água. Sealapex e Biodentine apresentaram a maior perda de volume. O tamanho das amostras não afetou a porcentagem de alteração volumétrica dos materiais. **Subprojeto 3-** avaliou escoamento e preenchimento volumétrico dos materiais usando diferentes modelos de teste. Biodentine apresentou menor escoamento e melhor preenchimento que IRM quando avaliado no modelo com maior altura, enquanto MTA apresentou o maior escoamento neste modelo. Não houve diferença no escoamento e preenchimento proporcionados pelos cimentos obturadores nos diferentes modelos e metodologias. Conclui-se que canaletas com maior altura e materiais com maior escoamento proporcionam menor capacidade de preenchimento para cimentos retrobturadores, enquanto que para os cimentos obturadores não houve influência dos modelos. **Subprojeto 4-** avaliou o efeito da imersão em água destilada e PBS na solubilidade e alteração volumétrica de cimentos endodônticos à base de silicato de cálcio (TotalFill BC Sealer, Sealer Plus BC e Bio-C Sealer), em comparação ao AH Plus. Os cimentos de silicato de cálcio apresentaram maior solubilidade e alteração volumétrica quando imersos em água destilada, mostrando maiores valores que AH Plus. **Subprojeto 5-** avaliou MTA, IRM e Biodentine, após preenchimento de retrocavidades em pré-molares superiores. **5A-** Foram avaliados estabilidade volumétrica e morfológica, porosidade e vazios na interface material/dentina antes e após imersão em PBS. Todos os materiais apresentaram estabilidade volumétrica. Biodentine apresentou redução dimensional e aumento na porosidade e espessura de vazios após a imersão. Conclui-se que MTA apresentou estabilidade dimensional e volumétrica, apesar de maior porosidade que Biodentine. IRM apresentou estabilidade após imersão. **5B-** Avaliou o impacto do tamanho do voxel (5, 10 e 20 μm) na avaliação por micro-CT da interface material/dentina e espessura dos cimentos, além da aplicação de diferentes softwares na análise do volume e porosidade dos materiais nos tamanhos de voxel citados. Foram observados aumento de volume e espessura, além de redução na porosidade e vazios na interface quando os materiais foram avaliados a 20 μm. Quando comparados os softwares, Biodentine mostrou diferenças para avaliação de volume e porosidade em todos os tamanhos de voxel. Conclui-se que maiores resoluções e radiopacidade dos materiais aumentaram a similaridade entre os softwares e a confiabilidade dos resultados.

Palavras chave: Endodontia. Materiais dentários. Propriedades físicas. Propriedades químicas. Microtomografia por Raio-X.

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ABSTRACT

This study evaluated new proposals and the necessary parameters for physicochemical analysis of materials using micro-CT. The materials used in subprojects 1, 2, 3 and 5 were: AH Plus, Fill Canal and Sealapex; Biodentine, IRM and MTA. The ANOVA/Tukey and T tests (α = .05) were applied. **Subproject 1-** evaluated by micro-CT the effect of immersion time in distilled water (7 and 30 days) on the volumetric change of endodontic materials inserted in simulated cavities in resin molds. All root canal sealers showed a difference in the percentage of volumetric change from 7 to 30 days of immersion. It is concluded that longer immersion periods can influence the volumetric changes of materials. **Subproject 2-** evaluated the influence of the size of specimens (thickness of 1.50 mm and internal diameters of 6.30, 7.75 and 9, 00 mm) in the volumetric change of materials after immersion in water. Sealapex and Biodentine showed the greatest volume loss. The sample size did not affect the percentage of volumetric change in the materials. **Subproject 3-** evaluated flow and volumetric filling of materials using different test models. Biodentine showed less flow and better filling than IRM when evaluated in the model with the highest height, while MTA presented the highest flow in this model. There was no difference in the flow and filling provided by the root canal sealers in the different models and methodologies. It is concluded that grooves with higher height and materials with greater flow provide less filling capacity for root-end filling cements, while for root canal sealers there was no influence of the models. **Subproject 4-** evaluated the effect of immersion in distilled water and PBS on the solubility and volumetric change of calcium silicate-based endodontic sealers (TotalFill BC Sealer, Sealer Plus BC and Bio-C Sealer), compared to AH Plus. Calcium silicate-based sealers showed greater solubility and volumetric changes when immersed in distilled water, showing higher values than AH Plus. **Subproject 5-** evaluated MTA, IRM and Biodentine, after root-end filling in maxilary premolars. **5A-** Volumetric and morphological stability, porosity and voids at the material/dentin interface were evaluated before and after immersion in PBS. All materials showed volumetric stability. Biodentine showed a dimensional reduction and an increase in the porosity and thickness of voids after immersion. It is concluded that MTA presented dimensional and volumetric stability, despite greater porosity than Biodentine. IRM showed stability after immersion. **5B-** Evaluated the impact of the voxel size (5, 10 and 20 μm) in the micro-CT evaluation of the material/dentin interface and cement thickness, in addition to the application of different software packages on analysis of volume and porosity of the materials in these cited voxel sizes. Increased volume and thickness were observed, as well as reduced porosity and voids at the interface when the materials were evaluated at 20 μm. When comparing the software packages, Biodentine showed differences for the evaluation of volume and porosity in all voxel sizes. It is concluded that higher resolution and radiopacity of the materials increased the similarity between the software and the reliability of the results.

Keywords: Endodontics. Dental Materials. Physical properties. Chemical properties. X-Ray microtomography.

SUMÁRIO

1 INTRODUÇÃO

Materiais endodônticos devem apresentar propriedades físico-químicas de acordo com normas e testes padronizados definidos pela ANSI-American National Standards Institute / ADA -American Dental Association (ADA)[1] e ISO - International Organization for Standardization[2], que estabelecem metodologias padronizadas para análise do tempo de presa, escoamento, espessura de filme, solubilidade, radiopacidade, estabilidade dimensional e resistência à compressão para cimentos.

O escoamento dos materiais endodônticos é necessário para o preenchimento do canal radicular ou cavidade retrógrada[3,4]. O teste de avaliação determinado pela ISO 6876[2] é realizado por meio da colocação de 0,05 ± 0,005 mL do material no centro de uma placa de vidro. Após 180 ± 5 s deve-se colocar uma segunda placa de vidro, seguido por um peso proporcionando massa total na placa de 120 ± 2 g. Dez minutos após o início da mistura o diâmetro máximo e mínimo do disco de cimento formado é mensurado. Se a diferença entre esses diâmetros for menor que 1 mm, a média dos diâmetros é anotada. Esta estabelece que cimentos endodônticos devem apresentar diâmetro superior a 17 mm de escoamento. Entretanto, a metodologia proposta pela ISO não fornece correlação entre escoamento e capacidade de preenchimento dos materiais[5].

A solubilidade dos cimentos obturadores é uma propriedade importante uma vez que a dissolução do material pode favorecer infiltração, comprometendo o sucesso do tratamento[6]. De acordo com os padrões estabelecidos pela ISO 6876[2] ou ANSI/ADA nº 57[1], os cimentos devem apresentar valores inferiores a 3% de solubilidade. A avaliação da solubilidade é realizada pela diferença de massa, em gramas, antes e após a imersão em água[7] após um período de 24 horas. No entanto, períodos maiores de análise são utilizados com o intuito de representar o comportamento dos materiais ao longo do tempo[8-14].

A estabilidade dimensional é também uma propriedade essencial para os materiais endodônticos, visto que a contração pode favorecer infiltração de microrganismos e seus produtos tóxicos, comprometendo o selamento endodôntico[15]. Embora a avaliação desta propriedade tenha sido removida da norma ISO 6876/2012, as especificações ISO 6876/2002 indicam que a alteração dimensional não deve exceder 1,0% em contração ou 0,1% em expansão. O teste utiliza corpos de prova com 12 mm de altura e 6 mm de diâmetro, preenchidos com

2 g de cimento. Os corpos de prova são mensurados com paquímetro digital e posteriormente acondicionados em frascos com água destilada por 30 dias. Após este período de tempo, as amostras são removidas dos recipientes, secas em papel absorvente e novamente medidas.

A ISO 6876 para estabilidade dimensional preconiza mensuração linear, em milímetros, com acurácia de avaliação de ±1 μm, podendo ser insuficiente para registrar pequenas alterações, além da possibilidade dos materiais contraírem ou expandirem para todas as direções[16]. Ainda, a solubilidade é avaliada pela diferença de massa, em gramas, antes e após a imersão em água, sendo que os materiais podem absorver água ou apresentar desintegração durante o armazenamento[7], levando à necessidade de desenvolvimento de metodologias de avaliação de alterações de volume.

Meios alternativos são propostos para avaliação da solubilidade e alteração dimensional com menor dimensão dos corpos de prova. Carvalho-Junior et al.[17], propuseram amostras com dimensões menores para estas análises reduzindo o material necessário para o experimento, sem afetar a acurácia do método avaliado. Os autores observaram correlação entre os resultados e concluíram que a redução do material das amostras não afeta a precisão dos métodos testados.

Cimentos à base de óxido de zinco e eugenol foram introduzidos na endodontia por Grossman, em 1936, para serem usados juntamente com a guta-percha na obturação de canais radiculares. Apresentam tempo de presa e escoamento aceitáveis[18,19], pequena alteração dimensional[20,21], radiopacidade, espessura de filme adequada[21], além de efeito antimicrobiano[22,23]. Todavia, a solubilidade destes cimentos mostra valores acima do recomendado[20,21].

AH Plus® (Dentsply, DeTrey GmbH, Konstanz, Alemanha) é um cimento à base de resina epóxi que tem sido usado como padrão ouro para comparações com cimentos endodônticos[21]. Análises realizadas segundo metodologias determinadas pela ISO 6876 têm mostrado que este cimento apresenta baixa solubilidade[24-32] e alteração dimensional[18,28,33], adequado tempo de presa[24,29-31], escoamento[24,27-29,31], penetrabilidade nos túbulos dentinários[34] e ótima radiopacidade[25-27,29,30].

Sealapex (SybronEndo, Orange, CA, USA) é um cimento à base de hidróxido de cálcio que apresenta adequada propriedade biológica[35], capacidade de selamento[36,37], escoamento aceitável[27] e penetrabilidade nos túbulos dentinários[34], embora mostre alta solubilidade[27,38,39].

Cimentos à base de silicato de cálcio são indicados para uso na endodontia. Prati e Gandolfi[40] relatam que esses materiais apresentam presa e promovem selamento em ambientes úmidos, além de apresentarem propriedades mecânicas adequadas. Os cimentos de silicato de cálcio expandem em relação ao volume inicial, o que contribui para a capacidade de selamento[41]. Entretanto, alta solubilidade tem sido relatada para esses materiais[42,43].

EndoSequence e TotalFill BC Sealer (FKG Dentaire SA, La Chaux-de-Fonds, Suíça) são cimentos endodônticos biocerâmicos pré-misturados prontos para uso[44]. TotalFill BC Sealer apresenta adequadas propriedades físico-químicas, tais como tempo de presa, radiopacidade, escoamento e capacidade de alcalinização[45,46], além de propriedades biológicas[46,47] e efeito antimicrobiano[47].

Novos cimentos obturadores à base de silicato de cálcio foram lançados recentemente no mercado nacional. Dentre eles, Bio-C Sealer (Angelus, Londrina, PR, Brasil) e Sealer Plus BC (MK Life, Porto Alegre, RS, Brasil). Bio-C Sealer apresenta silicatos de cálcio, aluminato de cálcio, óxido de cálcio, óxido de zircônio, óxido de ferro, dióxido de silício e agente dispersante em sua composição. Estudos prévios mostraram que Bio-C apresenta tempo de presa curto, capacidade de alcalinização do meio, escoamento e radiopacidade adequados[43], além de citocompatibilidade[48]. Sealer Plus BC é um material composto por óxido de zircônio, silicato tricálcico, silicato dicálcico, hidróxido de cálcio e propilenoglicol. Sealer Plus BC apresenta adequadas propriedades de tempo de presa, pH, liberação de cálcio, escoamento e radiopacidade[49], bem como propriedades biológicas satisfatórias[50]. Alta solubilidade tem sido reportada para Bio-C e Sealer Plus BC[43,49].

MTA é um biomaterial à base de silicato de cálcio desenvolvido por Torabinejad et al.[51] para o tratamento de perfurações radiculares e como material retrobturador[52-56] que apresenta biocompatibilidade e capacidade de induzir a formação de tecido mineralizado[22,57]. MTA apresenta radiopacidade[58,59], alta capacidade de alcalinização do meio[58] e baixa solubilidade[13,59].

Biodentine (Septodont, Saint Maur des Fossés, France) é um biomaterial à base de silicato de cálcio com propriedades mecânicas semelhantes à dentina, que pode ser utilizado como um substituto da dentina, e com indicações similares ao MTA[60-66]. Biodentine consiste de um pó e um líquido. O pó contém silicato tricálcico e dicálcico, bem como carbonato de cálcio e óxido de zircônio como radiopacificador. O líquido é constituído por cloreto de cálcio, que é utilizado como um acelerador de presa e agente redutor de água, em solução aquosa com uma mistura de policarboxilato (um agente superplastificante)[60,65]. Biodentine apresenta melhor consistência de manipulação[67], entretanto, maior solubilidade que MTA[11,13,68].

Novas metodologias como a microtomografia computadorizada (micro-CT) podem ser usadas para análise de propriedades físico-químicas de cimentos endodôntico. Micro-CT é utilizado para avaliação da correlação entre adaptação marginal de cimentos e sua capacidade seladora[69,70], identificando a presença de espaços vazios e falhas entre o material e a superfície da raiz[71-73], sendo uma ferramenta de análise 3D não destrutiva para análise da microestrutura interna de materiais obturadores na interface com a dentina[74]. A utilização do micro-CT possibilita também análise volumétrica (em mm³) dos materiais, em diferentes períodos, sendo possível correlacionar esta propriedade com a solubilidade[6] e alteração dimensional dos cimentos[13,43,45,75-77]. Trata-se de uma técnica não invasiva, com muitas aplicações na Endodontia[74,78,79].

A partir de nova metodologia desenvolvida, a avaliação do escoamento por meio do uso de micro-CT mostrou vantagens na avaliação da capacidade de escoamento e preenchimento em volume de espaços laterais, característica importante para um cimento endodôntico. Tanomaru-Filho et al.[80] avaliaram o escoamento e preenchimento de cimentos endodônticos (MTA, Biodentine e óxido de zinco e eugenol) em micro-CT. Foi confeccionada placa de vidro com uma cavidade central e 4 canaletas, no sentido horizontal e vertical, a partir dessa cavidade. Os cimentos reparadores foram colocados sobre a cavidade central e sobre eles nova placa de vidro (20 g) e metal (100 g) com massa total de 120 g, de maneira similar ao teste convencional proposto pela ISO 6876[2]. A avaliação foi realizada em micro-CT com relação à mensuração do escoamento linear (mm) do material em cada lado da canaleta (horizontal e vertical) além do preenchimento em volume (mm³) dos materiais nas áreas central e laterais. Os autores observaram ausência de correlação entre escoamento e preenchimento. Embora o estudo tenha demonstrado a importância de novas metodologias utilizando micro-CT para avaliação dessas propriedades, as dimensões ideais para a confecção desse dispositivo precisam ser definidas melhorando a precisão e diferenciação entre materiais. Além disso, a aplicação desta metodologia para avaliação de cimentos obturadores ainda não foi realizada.

Para análise da alteração volumétrica em micro-CT, Torres et al.[13] utilizaram modelos transparentes à base de resina acrílica confeccionados por meio de moldes metálicos com cavidades de 3 mm de profundidade e 1 mm de diâmetro. Os autores observaram que este teste permitia a avaliação concomitante das propriedades de solubilidade e alteração dimensional. Entretanto, os diâmetros dos corpos de prova podem ser alterados a fim de observar a interação dos materiais com o meio aquoso e suas consequências. Para avaliação da alteração no volume dos materiais, o escaneamento das amostras é realizado inicialmente após a presa dos materiais e após imersão em água destilada. Desta forma, podemos considerar também que a influência do tempo de imersão das amostras na estabilidade volumétrica dos materiais ainda não está definida.

A aquisição das imagens e programas de análise em micro-CT são variadas, sendo que a variação de voxel pode gerar variabilidade de resultados por diferenças de análise. Além disso, variações de parâmetros e programas de análise podem influenciar a obtenção dos dados e precisão dos métodos para cada finalidade. A posterior análise das imagens obtidas também apresenta variação. O uso de softwares específicos do microtomógrafo utilizado ainda se apresenta como a principal ferramenta de análise[71,81-85]. Porém, outros softwares são propostos[86-88], podendo proporcionar novas formas de análises.

Desta forma, este projeto visa realização de diferentes testes com corpos de prova variados, utilizando testes convencionais e micro-CT, a fim de desenvolver metodologias precisas e detalhadas para avaliar algumas propriedades físico-químicas de materiais endodônticos, bem como a definição de protocolos mais adequados para as diferentes avaliações.

2 PROPOSIÇÃO

Desenvolver metodologias empregando micro-CT para avaliação de solubilidade, escoamento e alterações morfológicas de materiais endodônticos, além da avaliação comparativa de dados obtidos em diferentes resoluções e programas de análise visando a definição de protocolos adequados para as diferentes análises.

3 PUBLICAÇÕES

Este trabalho foi dividido em oito artigos, sendo que parte deles foi publicado e parte foi submetida para publicação.

3.1 Publicação 1*

Effect of immersion time in distilled water on volumetric stability of different endodontic materials

ABSTRACT

Objective: Endodontic materials should present volumetric stability over time. The aim of this study was to evaluate the volumetric change of the root canal sealers AH Plus, Fill Canal, and Sealapex; and the root-end filling materials Biodentine, IRM, and MTA after different time periods of immersion in distilled water. **Methodology:** Resin models were manufactured with cavities 3 mm deep. The cavities were filled with the materials and scanned by microcomputed tomography (micro-CT) after setting. After 7 and 30 days immersed in distilled water, the filled cavities were scanned again to evaluate the volumetric change of the materials. Statistical analysis was performed by ANOVA, Tukey and t-test with 5% significance level. **Results:** All the root canal sealers showed difference in their percentage of volumetric change from 7 to 30 days of immersion ($p<0.05$). After 30 days, Sealapex presented greater volumetric change than the other sealers ($p<0.05$). Among the root-end filling materials, only IRM showed difference between 7 and 30 days of immersion ($p<0.05$). MTA presented the lowest, and Biodentine the highest volumetric change ($p<0.05$). **Conclusions:** The immersion time can affect the volumetric stability of AH Plus, Sealapex, Fill Canal, and IRM. Although Biodentine showed more volumetric changes than MTA at all periods, both materials kept their volume from 7 to 30 days.

Keywords: Dental materials. Endodontics. Physical properties. X-Ray microtomography.

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* Artigo escrito nas normas do periódico *Journal of Applied Oral Science*, ao qual foi submetido. A política de copyright e autoarquivo de editores para esta revista está disponível no Anexo B.

INTRODUCTION

Dental materials should present proper physicochemical properties. Standardized tests are defined by the American Dental Association (ADA)[1] and International Organization for Standardization (ISO)[2] for solubility and dimensional stability analyses of root canal sealers[3].

Endodontic materials may not have high solubility, which could allow leakage, leading to an unsuccessful treatment[4]. Their solubility is assessed by the difference in mass before and after immersion in distilled water for 24 hours, according to ISO[2] and ADA[1] standards. However, the difference between initial and final weight could not be enough to evaluate the volumetric performance of a dental material[5-7]. The dimensional stability is another important physical property, since shrinkage may also lead to leakage and a fail in the endodontic sealing[8].

Microcomputed tomography (micro-CT) has been used as a complementary tool to evaluate the solubility and dimensional stability of endodontic materials by the volumetric change analysis after immersion of the materials in a fluid[5-7,9,10]. Micro-CT is better than conventional methods due to its nondestructive characteristic[11]. Therefore, it is possible to analyze the same specimen over time[5,6,10]. In order to evaluate the volumetric change of materials by micro-CT, cavities have been filled with the cements, decreasing the contact of the materials with the fluid to allow a methodology closer to a clinic condiction[4-6,9,10].

Aiming to evaluate the materials behavior, the solubility of endodontic cements has been evaluated for longer periods, and a previous study[12] showed that root canal sealers based on calcium silicate or salicylate resin showed high solubility during 6 months of evaluation. This kind of assessment over time shows that some materials could not be stable[13], which may compromise the sealing and endodontic treatment success[8]. Most studies evaluated the volumetric stability of endodontic materials after 7 days of immersion[4-7,9,10,14]. Although some studies have been evaluated the materials after 30 days[5,6,10,14,15], the authors did not perform a comparison between the time periods. Therefore, the comparison by micro-CT of the volumetric stability of endodontic materials based on several compositions after different periods of immersion seems relevant.

The aim of this study was to compare by using micro-CT the volumetric stability of different endodontic cements and root canal sealers after 7 and 30 days of immersion in distilled water. The null hypothesis was that there is no effect of immersion time in distilled water on the volumetric change of the materials.

MATERIAL AND METHODS

The materials evaluated, their manufacturer, composition and proportion used are described in Table 1.

Volumetric change evaluation

The evaluation of the volumetric change of the materials was performed based on a previous study[6]. Cavities in acrylic resin measuring 3 mm deep were manufactured using metal molds (n = 12). The cavities were filled with each material and radiographed using a digital X-ray (Kodak RVG 6100 Digital Radiography System, Marne-la-Vallée, France) to show their complete filling with the materials. The samples were kept in an oven at 37ºC and 95% humidity for 2 days to the root-end filling materials and 7 days when using the root canal sealers. The scanning procedure was performed by micro-CT SkyScan 1176 (Bruker, Kontich, Belgium) after setting and after 7 and 30 days of immersion of the specimens in distilled water. The following scanning parameters were used: 50 kV power, 500 µA energy, aluminum filter of 0.5 mm, 18 µm voxel size, and an evolution cycle of 360°. The images were reconstructed using NRecon software (V1.6.4,7; Bruker), superimposed with geometric alignment using the Data Viewer software (V1.5.1, Bruker), and quantitative analyses were performed using CTAn software (V1.14.4, Bruker). The total volume of the cavities filled with each material was quantified and the volumetric chance was considered the difference between the baseline volume values and the values of the experimental periods of 7 and 30 days.

Statistical analysis

All data were analysed with the GraphPad Prism 7.00 (GraphPad Software, La Jolla, CA, USA) statistical software package. Data were submitted to normality test, and One-way ANOVA/Tukey tests were used for statistical differences between the endodontic materials. T-testing was used for comparison of the changes at the time points. The level of significance was set at 5%.

RESULTS

The results regarding the evaluation of the root canal sealers are showed in Table 2. All materials had difference in their percentage of volumetric change from 7 to 30 days of immersion ($p<0.05$). AH Plus and Fill Canal presented volume gain, while Sealapex had a volume loss after 7 days ($p<0.05$). After 30 days, all sealers had volume loss, and Sealapex presented greater volumetric change than the other materials ($p<0.05$).

When evaluating the root-end filling materials, only IRM showed difference between 7 and 30 days of immersion ($p<0.05$). MTA presented the lowest and Biodentine the highest volumetric change ($p<0.05$). The results are presented in Table 3. Representative models of volume gain and volume loss after immersion are showed in Figure 1.

DISCUSSION

Although the ISO/ADA standards indicate materials solubility evaluation after 24 hours of immersion, longer periods have been used[5-7,12,14-21]. These longer periods of assessment are important since some materials could maintain their solubilization over time[12,13], which could compromise the sealing of the root canals and the endodontic treatment longevity[8].

In order to complement the conventional tests, the current study evaluated the volumetric stability of endodontic materials by means of a non-destructive tridimensional approach[9], which allowed following the behavior of endodontic materials based on different components after 7 and 30 days of immersion. Between the scanning periods, interaction of endodontic materials with the aqueous medium may occur, mainly for hydrophilic materials[8]. Therefore, an effect of the immersion time on the volumetric stability was observed for all the root canal sealers evaluated besides the root-end filling material IRM, rejecting our null hypothesis.

AH Plus showed a volume increase after 7 days of immersion and a slight volume loss after 30 days. On the other hand, Sealapex had volume loss in both periods, with the highest values after 30 days. The results observed after 7 days for AH Plus occurred probably because of its low solubility associate with its expansion[3]. Our findings corroborate Silva, et al.[22] (2016), which evaluated the solubility of AH Plus after 1, 7, 14 and 28 days of immersion. The authors observed an increase in the solubility of this sealer over time, from 0.41% at 7 days to 0.71% of mass loss after 28 days. Although an effect of the immersion time has been observed, the values were below 1%, showing the stability of AH Plus, in agreement with previous studies[12,13]. This stability could be justified by the strong cross-links in the resinous polymers of AH Plus[3]. Regarding Sealapex, the solubility and shrinkage previously observed for this sealer[3] could contribute for the result of volume loss. Sealapex has a complex and quite inhomogeneous setting reaction, with the presence of porosities that can increase the solubilization of this sealer[23]. The presence of bismuth oxide as a radiopacifying agent can also contribute to its integrity reduction[24,25].

Some characteristics of zinc oxide and eugenol-based materials and their interaction with a fluid medium such as the properties of wash-out, sorption and fluid uptake were few investigated[26]. However, greater solubility[27] and infiltration[28] have been observed for these materials when in comparison with resin-based materials. Fill Canal and IRM are zinc oxide and eugenol-based materials. Both materials had a volume gain after 7 days, while after 30 days a volume loss was observed for them. The initial volumetric increase for IRM and Fill Canal could be related to the interaction of the aqueous medium with the polymers in the composition of these materials, such as poly methyl methacrylate and hydrogen resin, respectively. After 30 days a leaching effect of eugenol could lead to disintegration and volume loss[29].

Biodentine and MTA are calcium silicate-based cements that present bioactivity, influencing the repair of adjacent tissues[30]. According to our results, both materials kept their volume loss from 7 to 30 days. A previous study[31] investigated the solubility of MTA since its mixture until 672 hours, and observed values above the 3% weight lost considered acceptable by the ISO standard. However, the authors also stated a high initial solubility followed by a constant decrease over time for MTA[31]. This statement corroborates another long-term investigation regarding MTA solubility[32], which demonstrated that MTA is soluble over time but with a decreasing rate. This previous study also allege that MTA is mainly composed by an insoluble matrix of silica that maintains its integrity even in contact with water, besides the capacity of MTA to promote a high pH that was maintained in the aqueous environment over time[32]. Our study did not consider only the mass loss of MTA, but its complete volumetric behavior, which is in agreement with previous findings showing the volumetric stability of MTA[10]. This result suggest that water uptake may compensate the solubility of MTA, since its hydration process promotes an increase in volume[31].

Biodentine showed greater volume loss than MTA. The solubility of Biodentine has been reported[6,10,20,33,34]. However, there is no long-term investigation regarding Biodentine solubility. Moreover, for calcium silicate-based cements, the solubilization of their components may have biological relevance[13], favoring ion release and bioactive potential with formation of apatite[35]. Biodentine also has expansion[6] and fluid uptake[20], which could compensate its volume loss. Furthermore, when characterizing and investigating the hydration of Biodentine and MTA Angelus, Camilleri, et al.[36] (2013) showed that Biodentine resulted in a material with enhanced chemical properties, while the clinker of MTA Angelus was incompletely sintered leading to a variability in its mineralogy, besides less amount of tricalcium silicate generating a slower reaction rate. Therefore, although Biodentine showed a higher volume loss than MTA in both periods evaluated, MTA and Biodentine had volumetric stability between 7 and 30 days.

Based on the results of the current study, the volumetric stability of the materials depends on their basic composition. Regarding the root canal sealers, although AH Plus and Fill Canal were affected by the immersion time, their changes were below 1%. Sealapex had higher volume loss than the other sealers, with greater values after 30 days. However, its solubilization could promotes calcium and hydroxyl ions release, which may aid in the bioactivity of this sealer[35]. Among the root-end filling materials, MTA had the greatest volumetric stability.

CONCLUSIONS

A greater immersion time in distilled water affected the volumetric changes for AH Plus, Sealapex, Fill Canal and IRM. Although Biodentine had higher changes than MTA, both materials kept their values between 7 and 30 days. The greatest volumetric loss occurred for Sealapex among the root canal sealers and Biodentine among the root-end filling materials. Further studies comparing volumetric changes over longer periods should be performed in order to complement our findings regarding the behavior of endodontic materials, providing a long-term success of endodontic therapies.

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The authors declare that they have no conflict of interest.

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TABLES

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

Material	Manufacturer	Composition	Proportion
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 gotas
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

Table 2 - Volumetric change values (%) after 7 and 30 days (mean and standard deviation) observed in root canal sealers

Volumetric Change (%)	AH Plus	Fill Canal	Sealapex
7 days	0.65 (0.26)^{A,b}	0.89 (0.19)^{A,c}	-0.48 (0.18)^{A,a}
30 days	-0.32 (0.11)^{B,b}	-0.42 (0.11)^{B,c}	-1.18 (0.49)^{B,a}

^{AB}Different capital letter in the same column indicate statistically significant difference between the timepoins (p<0.05)

^{abc}Different lower case letters on the same row indicate statistically significant difference between the sealers (p<0.05)

Negative values in the volumetric change test indicate volume loss

Table 3 - Volumetric change values (%) after 7 and 30 days (mean and standard deviation) observed in endodontic cements

Volumetric Change (%)	Biodentine	IRM	MTA
7 days	-2.19 (0.76)^{A,a}	0.77 (0.18)^{A,b}	-0.51 (0.23)^{A,c}
30 days	-2.07 (0.66)^{A,a}	-1.46 (0.47)^{B,b}	-0.49 (0.20)^{A,c}

^{AB}Different capital letter in the same column indicate statistically significant difference between the timepoins (p<0.05)

^{abc}Different lower case letters on the same row indicate statistically significant difference between the cements (p<0.05)

Negative values in the volumetric change test indicate volume loss

FIGURE

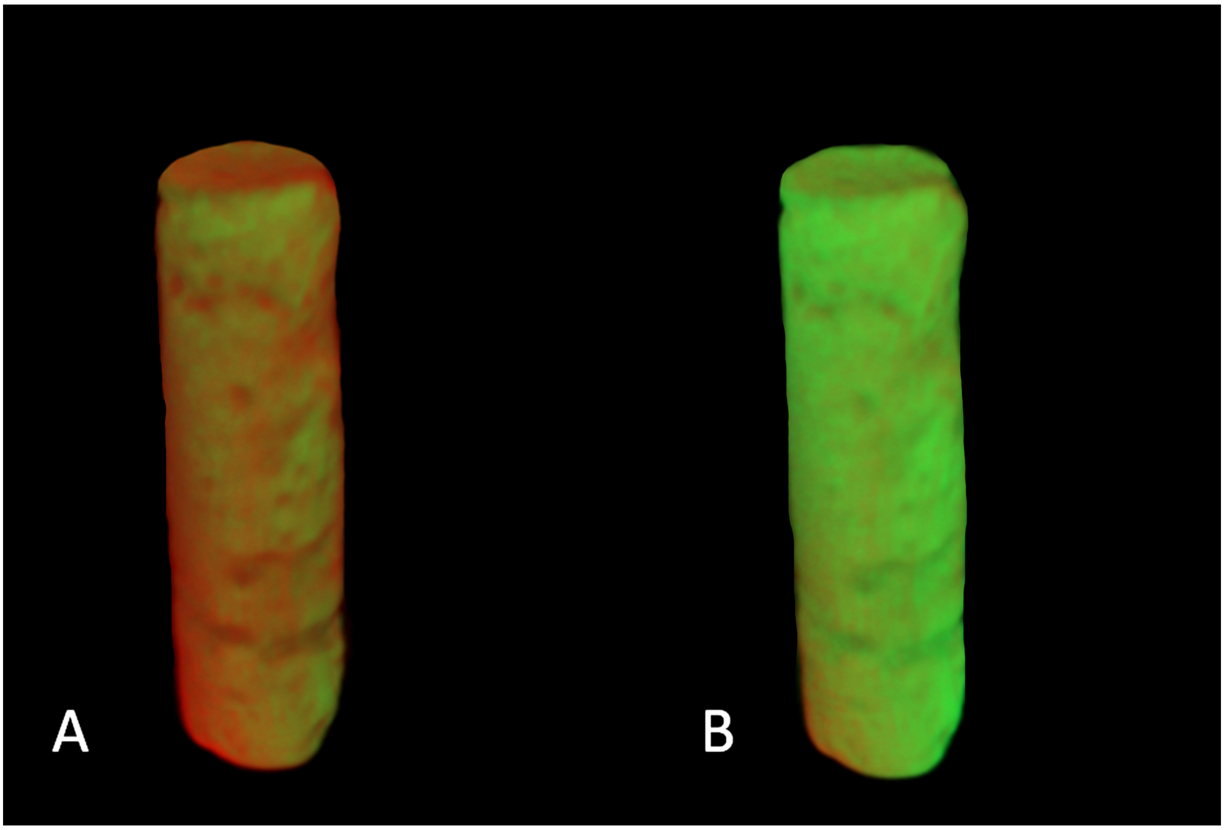

Figure 1 – 3D models created in CTVox software illustrating the volumetric change before (green) and after (red) immersion in distilled water. A: representative image of volume gain after immersion. B: representative image of volume loss after immersion.

3.2 Publicação 2*

Effect of different test sample sizes on the volumetric change assessment of endodontic materials

SUMMARY

New methodologies using micro-CT to evaluate solubility besides dimensional and morphological changes of endodontic materials have been proposed. However, there is no standardization in the methods. The aim of this study was to assess the effect of the test sample size 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 1.50 mm and different diameters were manufactured: 6.30, 7.75, and 9.00 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 6.30 and 9.00 mm can be used to assess the stability of endodontic materials using micro-CT without affecting the percentage of volumetric change.

Key Words: Endodontics, physicochemical properties, root canal filling material, x-ray microtomography.

* Artigo escrito nas normas do periódico *Brazilian Dental Journal*, ao qual foi submetido. A política de copyright e autoarquivo de editores para esta revista está disponível no Anexo C.

INTRODUCTION

Root canal sealers associated to gutta-percha cones are requested to a tridimensional filling of the root canals, preventing leakage and promoting repair of periapical tissues (1). Besides that, endodontic cements are indicated for root-end filling, pulp capping, root and coronal perforation repairs, and regeneration (2).

An ideal endodontic material should be insoluble in tissue fluids, and dimensionally stable (3), since solubility and dimensional changes of the materials might compromise the sealing ability of root canal obturation leading to reinfection (4, 5). Therefore, more investigation about the different cements currently available are necessary to select materials based on their stability (6).

Many differences in the solubility test may be observed and the discrepancy of results could be related to their methodology (7). Besides that, only the solubility of a sealer may not be related to its dimensional stability and sealing ability (3). Moreover, the solubility of a sealer can be compensated by its fluid absorption (8).

Micro–computed tomographic (micro-CT) is a high-resolution technology that allows a nondestructive imaging analysis (9). Micro-CT is able to develop accurate tridimensional models with repeated exposures and acquisition of information (9). The use of micro-CT has increased in dentistry research (10). 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 (8, 11-13).

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. This gap in the literature leads us to question the influence that the amount of material that is exposed to the fluid would have on the solubilization and dimensional change of the samples.

Therefore, the aim of this study was to evaluate the effect of the test sample size 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 sample sizes.

MATERIALS AND METHODS

Specimen preparation

Based on a previous study (5), circular plastic molds measuring 1.5 mm thickness and 6.30 mm in diameter, 1.5 mm thickness and 7.75 mm in diameter, and 1.5 mm thickness and 9.00 mm in diameter were placed on a glass plate covered with cellophane film. The molds were randomly divided into 3 groups (n = 6), and filled with the endodontic materials (Table 1). 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 hours.

Volumetric change assessment

The volumetric change of the materials was evaluated according to a previous study (13). 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 5.00 mL of distilled and deionized water for the test samples with 6.30 mm in diameter, 7.50 mL for 7.75 mm, and 10.00 mL when using the test samples with 9.00 mm, and kept in an oven at 37°C for 7 days. After this period, the samples were placed in a desiccator under vacuum for 24 hours and scanned again. The reconstruction of the images was performed using NRecon software (V1.6.10.4; Bruker, Kontich, Belgium) with correction parameters for smoothing, beam hardening and ring artefacts. 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 of material to be calculated in mm^3. 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.

DISCUSSION

Physicochemical properties such as solubility and dimensional stability of the endodontic materials are related to their sealing ability (5). Thus, materials with low solubility and dimensional stability may decrease the possibility of gap formation between root canal dentine and the filling material (8).

According to the ISO or ANSI/ADA 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 (14). 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 (8, 11-13, 15). Thus, based on volume changes the stability of the materials can be evaluated (8).

Although several studies have been used 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 (16). This gap on the literature led to require researches to investigate the test variables to assess its influence on the results of endodontic studies (17).

Carvalho-Junior et al. (5) 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 corroborate their findings, since a variation in the diameter of the samples didn't interfere in the volumetric change results, accepting our null hypothesis.

Even using endodontic materials with different radiopacities, our study kept the same scanning parameters for all materials in order to standardize the test, since during the reconstruction of the images it is possible to decrease the artifacts for each material, without impacting the objective image analysis (10). Furthermore, the segmentation of the cement from the background is substantial for an accurate analysis, avoiding under or overestimation of the real volume (18). Thus, our analysis was performed using objective segmentation, which should be the first choice in micro-CT studies (18).

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 (8, 11-13). Moreover, AH Plus was the only sealer that showed a volume increase. The low solubility (11, 12) added to its dimensional expansion (12) could explain these findings. The strong cross-links in epoxy resin-based sealers justify the volumetric stability of AH Plus (19).

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 (20, 21). The presence of polycarboxylate in its composition presents a surfactant effect and may disperse the cement particles (20). The volume loss of Biodentine can also be associated with high amounts of ions release, an important factor for its bioactivity (21). The high volume loss in Sealapex can occur also due its solubility (11, 14), which is related to its non-homogeneous setting reaction, resulting in a fragile matrix (14, 19).

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 (12). However, the present study showed that the volumetric reduction was low, probably because eugenol is not water-miscible (15). Although with lower values than Biodentine, MTA also had a decrease in volume after immersion, as showed in previous studies (15, 22). 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 hours 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 (21). 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, it appears that the use of different sample sizes to assessment by micro-CT of the volumetric change of endodontic materials based on different components did not influence the outcomes. Thus, the diameter of the samples can be selected according to the researcher preference and should not affect the findings of the endodontic studies.

RESUMO

Novas metodologias utilizando micro-CT para avaliar a solubilidade além de alterações dimensionais e morfológicas de materiais endodônticos têm sido propostas. No entanto, não há padronização nos métodos. O objetivo deste estudo foi avaliar o efeito do tamanho dos corpos de prova na avaliação das alterações volumétricas de diferentes materiais endodônticos. Os cimentos obturadores AH Plus, FillCanal e Sealapex e os retrobturadores Biodentine, IRM e MTA foram utilizados nos testes. Foram confeccionadas amostras de cada material com uma espessura de 1,50 mm e diâmetros diferentes de: 6,30, 7,75 e 9,00 mm. As amostras foram digitalizadas 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 das amostras antes e após a imersão. Os dados foram submetidos aos testes ANOVA e Tukey ($p<0,05$). O tamanho das amostras não afetou a porcentagem de alteração volumétrica dos materiais ($p>0,05$). Todos os tamanhos de amostras apresentaram maior perda de volume para Sealapex entre os cimentos obturadores e Biodentine para os

retrobturadores ($p<0,05$). Como conclusão, Biodentine e Sealapex tiveram a maior perda de volume após imersão. Amostras com 1,5 mm de espessura e diâmetros variando entre 6,30 e 9,00 mm podem ser usadas para avaliar a estabilidade de materiais endodônticos usando micro-CT sem afetar a porcentagem de alteração volumétrica.

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DECLARATION OF CONFLICT OF INTEREST:

The authors declare that they have no conflict of interest.

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TABLES

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

Material	Manufacturer	Composition	Proportion
AH Plus	DentsplyDeTrey, Konstanz, Germar	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 gotas
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

Table 2 - Volumetric change values (%) using different test sample sizes (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 sample sizes ($p>0.05$)

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 test sample sizes (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 sample sizes ($p>0.05$)

Different lower case letters on the same row indicate statistically significant difference between the cements ($p<0.05$)

Negative values indicate volume loss

FIGURE

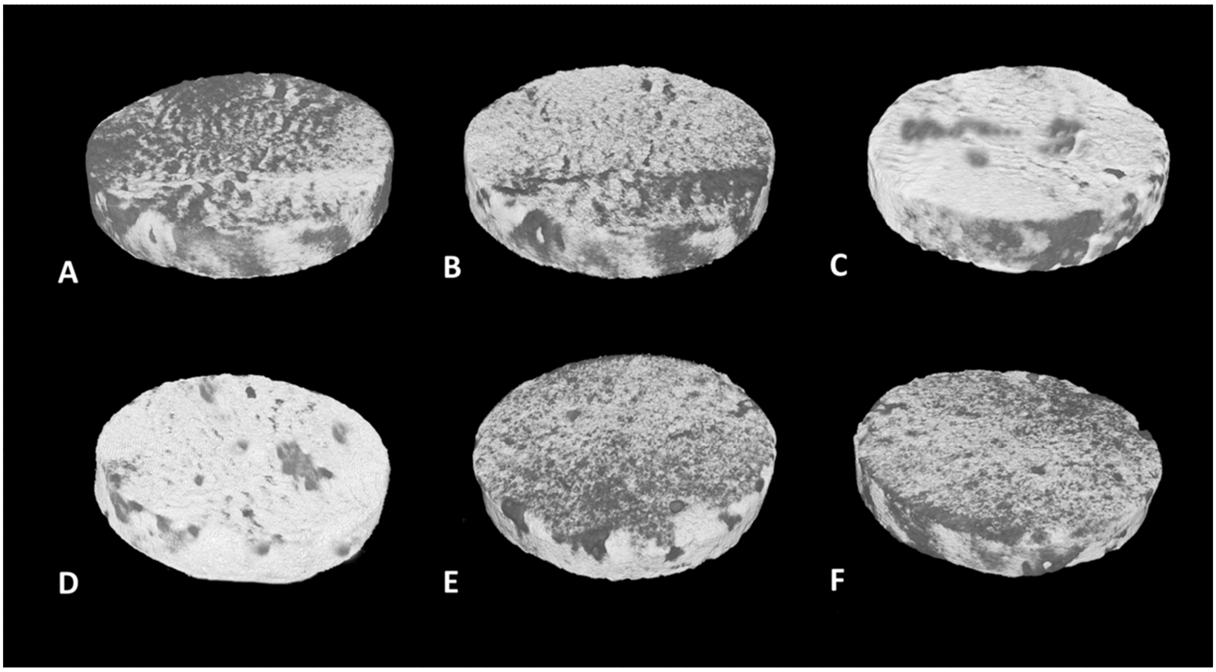

Figure 1 - 3D models representing the volumetric change assessment before (white) and after (grey) immersion of the samples in distilled water for AH Plus (A), Fill Canal (B), Sealapex (C), Biodentine (D), IRM (E), and MTA (F).

3.3 Publicação 3*

Micro-computed tomographic evaluation of the flow and filling ability of endodontic materials using different test models

* Artigo escrito nas normas do periódico *Restorative Dentistry & Endodontics*, no qual foi publicado (https://rde.ac/DOIx.php?id=10.5395/rde.2020.45.e11). A política de copyright e autoarquivo de editores para esta revista está disponível no Anexo D.

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Research Article

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Micro-computed tomographic evaluation of the flow and filling ability of endodontic materials using different test models

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

ABSTRACT

Objectives: This study compared the flow and filling of several retrograde filling materials using new different test models.
Materials and Methods: Glass plates were manufactured with a central cavity and 4 grooves in the horizontal and vertical directions. Grooves with the dimensions used in the previous study (1 × 1 × 2 mm; length, width, and height respectively) were compared with grooves measuring 1 × 1 × 1 and 1 × 2 × 1 mm. Biodentine, intermediate restorative material (IRM), and mineral trioxide aggregate (MTA) were evaluated. Each material was placed in the central cavity, and then another glass plate and a metal weight were placed over the cement. The glass plate/material set was scanned using micro-computed tomography. Flow was calculated by linear measurements in the grooves. Central filling was calculated in the central cavity (mm^3) and lateral filling was measured up to 2 mm from the central cavity.
Results: Biodentine presented the least flow and better filling than IRM when evaluated in the 1 × 1 × 2 model. In a comparison of the test models, MTA had the most flow in the 1 × 1 × 2 model. All materials had lower lateral filling when the 1 × 1 × 2 model was used.
Conclusions: Flow and filling were affected by the size of the test models. Higher grooves and materials with greater flow resulted in lower filling capacity. The test model measuring 1 × 1 × 2 mm showed a better ability to differentiate among the materials.

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

INTRODUCTION

An ideal retrograde filling material should be resistant to dislocating forces [1,2] to prevent leakage between the root canal system and periradicular tissues [3]. Furthermore, the filling and sealing ability of endodontic cement can affect the long-term outcomes of endodontic surgery [4-6]. Root-end fillings with zinc oxide and eugenol-based cements, as well as calcium silicate-based materials, have shown a high probability of success [7,8]. However, the use of biomaterials has been proposed as a way to achieve more predictable outcomes [5-7,9].

Author Contributions

Conceptualization: Torres FFE, Tanomaru-Filho M, Guerreiro-Tanomaru JM; Data curation: Torres FFE, Pinto JC; Formal analysis: Torres FFE, Tanomaru-Filho M; Funding acquisition: Torres FFE, Tanomaru-Filho M; Investigation: Torres FFE, Tanomaru-Filho M, Chavez-Andrade GM; Methodology: Torres FFE, Chavez-Andrade GM; Project administration: Tanomaru-Filho M; Resources: Tanomaru-Filho M, Guerreiro-Tanomaru JM; Software: Torres FFE, Pinto JC; Supervision: Tanomaru-Filho M; Validation: Torres FFE, Tanomaru-Filho M, Berbert FLCV; Visualization: Torres FFE, Tanomaru-Filho M, Berbert FLCV; Writing - original draft: Torres FFE, Tanomaru-Filho M, Guerreito-Tanomaru JM; Writing - review & editing: Tanomaru-Filho M, Torres FFE.

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The ability of endodontic materials to fill the spaces in the root canal in order to prevent fluid penetration is related to their flow [10]. However, flow and filling ability may not be directly proportional, considering that a material with a better ability to flow linearly will not necessarily provide greater filling capacity [11,12].

There are no previously established standards for evaluating the flow of root-end filling materials, and the International Standards Organization (ISO) standard [13] commonly used for the evaluation of root canal sealers does not document a correlation between the flow and fill properties of a material [12]. Thus, more accurate methods are required to evaluate the flow of endodontic materials [10]. For this reason, a novel test model was developed for concomitantly evaluating the flow and fill of endodontic cements using micro-computed tomography (CT) [12]. Micro-CT is a nondestructive, precise, and reproducible imaging tool that provides a 3-dimensional (3D) quantitative evaluation of filling materials [14].

The device developed to evaluate endodontic materials using micro-CT involves the delivery of endodontic cement between 2 glass plates with a metal weight over the top plate, with a similar design to that described in the ISO standards [13]. However, the bottom glass plate is manufactured with a central cavity and 4 grooves extending out horizontally and vertically. In this way, the model enables analysis of flow into the spaces and volumetric analysis, which allows the evaluation of the filling ability of a material in the central and lateral areas [12]. The proposed model using micro-CT assessment showed valid and reproducible results, and has the potential to improve flow analysis. Nevertheless, the influence of the size of the test models on the results has not yet been analyzed.

Since comparing different methods is essential for establishing the most suitable methodologies in endodontic research [15], the aim of this study was to investigate the influence of different sizes of test models on the linear flow and volumetric filling of Biodentine (Septodont, Saint-Maur-des-Fosses, France), intermediate restorative material (IRM; Dentsply DeTrey, Konstanz, Germany), and mineral trioxide aggregate (MTA; MTA-Angelus, Angelus, Londrina, PR, Brazil) using micro-CT. The null hypothesis was that there would be no differences in the flow and filling of the materials according to the test model used.

MATERIALS AND METHODS

Preparation of the test models

The first test model was manufactured according to the technique described by Tanomaru-Filho *et al.* [12]. A glass plate was fabricated with a central cavity (1 × 1 × 2 mm) (length, width, and height) and grooves extending out horizontally and vertically to the 4 sides. The other 2 dimensions were proposed to facilitate a comparison with the previous study, as follows: 1 × 1 × 1 mm and 1 × 2 × 1 (length, width, and height) (**Figure 1**). The samples

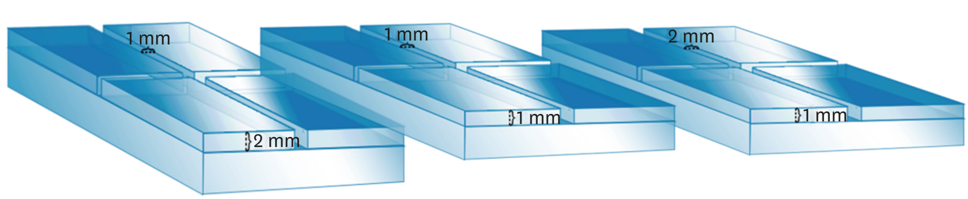

Figure 1. Illustration of the test models with a central cavity and lateral grooves manufactured with different dimensions: 1 × 1 × 2 mm, 1 × 1 × 1 mm, and 1 × 2 × 1 mm (length, width, and height).

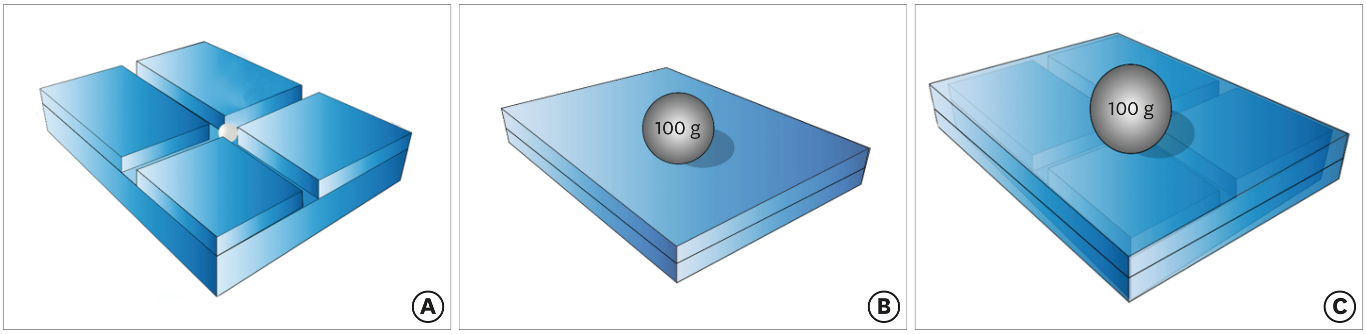

Figure 2. Illustration of the flow and filling ability evaluation process before the assessment using micro-computed tomography. The bottom glass plate with the endodontic cement placed in the central cavity (A). A view representing the assembled device, with the bottom glass plate, the top glass plate, and the metal weight over the cement (B). Another view representing the assembled device, using transparency to show the bottom plate and the metal weight over the material after flow inside the grooves (C).

were randomly divided into 3 groups ($n = 6$ each), according to the root-end filling material used. The complete information regarding the endodontic materials, their manufacturers, composition, and proportions is presented in **Table 1**. The procedure was performed by a single operator who was previously trained and calibrated. For each material, 0.050 mL was placed in the central cavity of the bottom glass plate, and another glass plate (20 g) and a metal weight (100 g) with a total mass of 120 g were placed on the materials and kept there for 10 minutes (**Figure 2**), according to the ISO 6876/2012 recommendation [13].

Micro-CT scanning and analysis

The glass plates/cement set was scanned with the SkyScan 1176 micro-CT system (SkyScan, Bruker, Kontich, Belgium). The micro-CT parameters were a voxel size of 9 µm, 90 kVp, 278 mA, a 0.1 mm copper filter, and 360° scanning. The linear flow (mm) measurement of the material on each side of the grooves (horizontal and vertical) was analyzed. The mean of the 4 measurements was considered the linear flow for each evaluation. The volume (mm^3) filled by the material in the central area was determined as the central cavity filling. The volume (mm^3) filled by the materials in the lateral areas was determined up to 2 mm on each side of the central cavity. The mean of the 4 measurements was considered as the lateral cavity filling for each analysis. The data sets were reconstructed using NRecon software (V1.6.10.4, Bruker). The correction parameters for smoothing, beam hardening, and ring artefacts were defined for each material. The flow into the grooves and the filling of the central and lateral cavities were calculated using the CTAn software (V1.15.4.0, Bruker). CTAn was also used to create 3D models of the materials, which were visualized using the CTVol program (V2.3.1.0, Bruker).

Table 1. Endodontic materials, their manufacturers, their composition, and the proportions used

Material	Manufacturer	Composition	Proportion
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 of powder to 6 drops of liquid
IRM	Dentsply, Caulk Milford, DE, USA	Powder: zinc oxide, polymethyl methacrylate Liquid: eugenol, acetic acid	1 g of powder to 0.2 mL of liquid
MTA-Angelus	Angelus, Londrina, PR, Brazil	Powder: tricalcium silicate, dicalcium silicate, tricalcium aluminate, calcium oxide, bismuth oxide Liquid: distilled water	1 g of powder to 0.33 mL of distilled water

IRM, intermediate restorative material; MTA, mineral trioxide aggregate.

Table 2. Mean and standard deviation of the results of flow (mm) and filling (%) of endodontic materials evaluated in test models with different sizes (length, width, and height)

Factor	Biodentine	IRM	MTA
Linear flow (mm)			
1 × 1 × 1 mm	6.70 ± 0.94^{Aa}	7.19 ± 0.39^{Aa}	6.85 ± 0.11^{Ba}
1 × 2 × 1 mm	6.13 ± 0.98^{Aa}	6.86 ± 0.53^{Aa}	6.55 ± 0.34^{Ba}
1 × 1 × 2 mm	5.95 ± 0.76^{Ab}	7.41 ± 0.54^{Aa}	8.53 ± 1.01^{Aa}
Central filling (%)			
1 × 1 × 1 mm	92.38 ± 8.13^{Aa}	84.93 ± 7.72^{Aa}	87.75 ± 6.56^{Aa}
1 × 2 × 1 mm	88.23 ± 4.14^{Aa}	85.34 ± 9.09^{Aa}	87.65 ± 12.62^{ABa}
1 × 1 × 2 mm	79.92 ± 4.84^{Aa}	61.09 ± 5.25^{Bb}	71.53 ± 9.06^{Bab}
Lateral filling (%)			
1 × 1 × 1 mm	94.67 ± 4.48^{Aa}	80.59 ± 2.63^{Ab}	80.88 ± 6.39^{Ab}
1 × 2 × 1 mm	85.32 ± 11.37^{ABa}	78.62 ± 3.44^{Aa}	72.62 ± 7.66^{ABa}
1 × 1 × 2 mm	73.04 ± 8.37^{Ba}	56.63 ± 2.04^{Bb}	60.42 ± 5.26^{Bab}

The values are mean ± standard deviation. Different lowercase letters on the same line indicate statistically significant differences between the different cements ($p < 0.05$). Different capital letters in the same column indicate statistically significant differences between the different test models ($p < 0.05$) (2-way analysis of variance and Tukey test).
IRM, intermediate restorative material; MTA, mineral trioxide aggregate.

Statistical analysis

The normality of the data was tested using the Kolmogorov-Smirnov test. The statistical analysis was performed with 2-way analysis of variance and the Tukey parametric test with a significance level of 5%.

RESULTS

The results are presented in **Table 2**. MTA and IRM presented similar linear flow and central cavity filling in all test models ($p > 0.05$). MTA had greater flow when using the test model with a height of 2 mm, with a higher value than Biodentine ($p < 0.05$). For central cavity filling, IRM and MTA showed worse results in the model with a height of 2 mm than in the model measuring 1 × 1 × 1 mm ($p < 0.05$). The same occurred for lateral cavity filling in all materials ($p < 0.05$). Biodentine showed better filling than IRM in the model with a height of 2 mm ($p < 0.05$). The calculation of the filling of the materials in the central and lateral areas, as well as a 3D model illustrating the flow and filling of the materials into the grooves, can be seen in **Figure 3**.

DISCUSSION

The flowability of endodontic sealers is evaluated using the ISO 6876/2012 standard [13] because a proper flow may allow filling irregularities [16]. However, this conventional test does not allow a 3D assessment of the filling ability of a material. Thus, it is not possible to determine whether proper flow is associated with adequate filling. Moreover, there is no standard for assessing the flow of root-end filling cements. Therefore, we evaluated the flow and filling of root-end filling materials using micro-CT according to Tanomaru-Filho *et al.* [12]. According to the authors, volumetric data reflect the ability of a material to fill a space and to flow into lateral spaces, an important property for endodontic materials. Furthermore, the proposed device allowed standardization of the amount of cement and the pressure to be used on the material during the test, similar to the device described in the ISO standard [13].

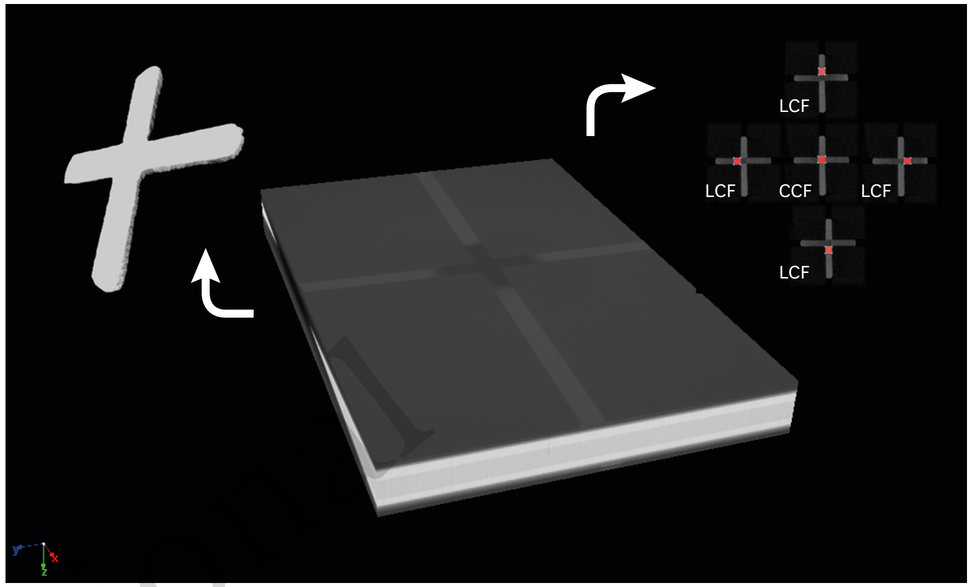

Figure 3. Illustration created in the CTVox software showing the assembled device composed of the bottom glass plate, the cement after flow inside the grooves, and the top glass plate during the scanning process on micro-computed tomography. The flow and filling representation were performed in 3 dimensions using the CTVol software. Central cavity filling (CCF) and lateral cavity filling (LCF) were evaluated using the CTAn software.

Numerous studies have proposed using micro-CT to complement conventional evaluations of endodontic materials [12,14,17-21]. However, since micro-CT is a relatively new methodology, these evaluations have lacked standardization. For this reason, it is necessary to evaluate the influence of the evaluation method on the results obtained.

Based on a new technique already recommended in the literature [12], the current study aimed to assess the influence of the dimensions of the test models on the flow and filling properties of different endodontic cements. Similar to previously observed results [11,12], there was no direct correlation between the properties evaluated, since when a material presented a greater flow, a proportional filling of the cavity was not observed. It was also found that the height of the test models had an influence on the results. When a height of 2 mm was used, MTA presented higher flow but less filling capacity. For Biodentine and IRM, lateral cavity filling was also lower when the highest model was used. In contrast, when the smallest dimensions were used, it was possible to observe greater flow and filling. These findings may be directly applied to clinical situations, as root canal systems present small irregularities to be filled by the material. Thus, our null hypothesis was rejected since the test models showed a direct influence on the results.

IRM is a zinc and eugenol-based cement that has been used for several decades. However, its cytotoxicity is an important limitation of this material [7]. Although Biodentine presents lower linear flow in the 2-mm-high model, it showed better filling than IRM. Therefore, it may be suggested that Biodentine has a better filling ability than IRM, which may be supported by previous results indicating that IRM showed high microleakage [22]. However, Tsesis *et al.* [23] evaluated the apical portion of root canals filled with MTA, IRM, and Biodentine regarding *Enterococcus faecalis* colonization and observed no differences among the root-end filling materials in the mean and maximal depths of bacterial colonization into the dentinal tubules. Those results suggest that similar success rates for root-end filling can be obtained using calcium silicate-based materials or cements based on zinc oxide and eugenol [8].

However, it is also important to consider that calcium silicate-based materials, such as MTA and Biodentine, are potentially bioactive when placed in direct contact with dentin, and may promote biomineralization [24]. This property enables a better seal for retrograde fillings [25], making them better options for the repair of furcal perforations [1]. Moreover, Akbulut *et al.* [26] observed no significant difference in the push-out bond strength of MTA-Angelus and Biodentine, in agreement with the similar characteristics observed for both materials in the present study.

Previous studies have evaluated the filling capacity of MTA and Biodentine using micro-CT [27-29]. This non-invasive imaging technique provides high-resolution images and enables a 3D volumetric analysis [30], allowing a material's filling ability to be evaluated in terms of the filling percentage [27,28]. Furthermore, the methodology employed in the current study allows a concomitant analysis of a material's flow rate and filling of lateral spaces [12], which is more difficult to achieve for root-end filling cements.

Regarding central cavity filling, our results showed that Biodentine and MTA presented similar values in all the test models. This finding corroborates the report of Küçükkaya *et al.* [27], who observed no significant difference between the obturation quality of MTA and Biodentine in terms of percentage volume of filling materials on micro-CT. The authors used tooth models that simulated perforating internal root resorption in the middle third of the root, and observed that the apical portion of the specimens presented a lower percentage of filling materials than the resorption cavities. Their result agrees with the present study, where the percentage of filling in the central cavity was greater than in the lateral spaces.

It is important to emphasize that when using the test model with the smallest dimensions, Biodentine showed better lateral filling than MTA. This finding may be related to the smaller particle size and higher surface area of Biodentine when compared to MTA. Moreover, the Biodentine radiopacifier is zirconium oxide, which has a smaller particle size and allows less porosity than the bismuth oxide used in MTA-Angelus [31]. Therefore, many studies have stated that a major disadvantage of MTA is its handling properties and its granular consistency. Consequently, additives have been proposed to increase its plasticity. Among these additives, methylcellulose, calcium chloride, calcium lactate gluconate, and propylene glycol improved the handling of MTA [32-34]. In Biodentine, a hydro-soluble polymer is incorporated to enhance its handling properties [31].

A recent systematic review was undertaken in order to evaluate the influence of variation in the design of the push-out bond test in endodontic research [35]. The authors concluded that standardization is required for future research, as well as accurate reporting of all test variables, to assess the impact of specific designs on endodontic evaluations. This statement reinforces the importance of conducting studies to establish methods for the reliable analysis of material properties.

CONCLUSIONS

The linear flow and filling ability of materials were affected by the size of the evaluated test models. Higher grooves and materials with higher flow showed a lower filling capacity. The test model measuring 1 × 1 × 2 mm showed the best ability to differentiate filling capacity among different materials.

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3.4 Publicação 4*

Evaluation of flow and filling of root canal sealers using different methodologies

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Original Article

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Evaluation of flow and filling of root canal sealers using different methodologies

Avaliação do escoamento e preenchimento de cimentos obturadores utilizando diferentes metodologias

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Resumo

Introdução: Escoamento e capacidade de preenchimento de cimentos obturadores são indispensáveis para um selamento hermético do canal radicular. Microtomografia computadorizada (micro-CT) pode ser utilizada como uma metodologia complementar para avaliação de tais propriedades. **Objetivo:** Avaliar escoamento e capacidade de preenchimento de AH Plus, Endofill e Sealapex, por meio de metodologia convencional e micro-CT. **Material e método:** O escoamento dos cimentos foi analisado de acordo com as normas ISO 6876/2012 e complementado pela avaliação em área. Placas de vidro foram confeccionadas nos diâmetros de 1×1×2 mm e 1×1×1 mm (comprimento, largura e altura), com uma cavidade central e quatro canaletas nas direções horizontal e vertical. Cada material foi colocado na cavidade central. Outra placa de vidro e um peso de metal foram colocados sobre o cimento e mantidos por 10 minutos. O conjunto placa de vidro/cimento foi escaneado usando micro-CT. O escoamento foi calculado por medição linear do material nas canaletas. O preenchimento (mm^3) central foi calculado na cavidade central e o preenchimento lateral foi medido até 2 mm a partir da cavidade central. Os dados foram submetidos aos testes ANOVA/Tukey ($\alpha=0.05$). **Resultado:** Todos os cimentos avaliados apresentaram escoamento de acordo com as normas ISO 6876. Os materiais mostraram capacidade de preenchimento da cavidade central superior a 80% e preenchimento lateral superior a 75%. Não houve diferença no escoamento (mm e mm^2) e na capacidade de preenchimento (mm^3) proporcionada pelos materiais ($p>0.05$). **Conclusão:** Todos os cimentos obturadores avaliados mostraram adequado escoamento e capacidade de preenchimento, sugerindo a aplicação clínica dos mesmos.

Descritores: Endodontia; materiais dentários; microtomografia por raio-X.

Abstract

Introduction: Flow and filling ability of root canal sealers are indispensable for hermetic sealing of the root canal. Micro-computed tomography (micro-CT) can be used as a complementary methodology to evaluate such properties. **Objective:** To evaluate the flow and filling ability of AH Plus, Endofill and Sealapex by conventional methodology and micro-CT. **Material and method:** The flow of the sealers was analyzed according to ISO 6876/2012 and complemented by the area evaluation. Glass plates were manufactured with diameters of 1×1×2 mm and 1×1×1 mm (length, width and height), with a central cavity and four grooves in the horizontal and vertical directions. Each material was placed in the central cavity. Another glass plate and a metal weight were placed on the cement and kept for 10 minutes. The glass plate/sealer set was scanned using micro-CT. The flow was calculated by linear measurement of the material in the grooves. The central filling (mm^3) was calculated in the central cavity and the lateral filling was measured up to 2 mm from the central cavity. Data were submitted to ANOVA/Tukey tests ($\alpha=0.05$). **Result:** All evaluated sealers presented flow according to ISO 6876 standards. The materials showed central cavity filling capacity higher than 80% and lateral filling greater than 75%. There was no difference in flow (mm and mm^2) and in the filling ability (mm^3) provided by the materials ($p>0.05$). **Conclusion:** All evaluated root canal sealers showed adequate flow and filling capacity, suggesting their clinical application.

Descriptors: Endodontics; dental materials; X-ray microtomography.

INTRODUCTION

A complete root canal filling is substantial for long-term success of endodontic treatment[1]. The obturation is classically performed using gutta-percha and different types of root canal sealers[2]. The root canal sealers fill the gaps between gutta-percha cones and the dentine walls[3]. Therefore, these materials should present adequate physical properties[4].

Among the important physical properties to a proper obturation, the flow of endodontic sealers allows their deeper penetrability into the irregularities of the root canal system, which contribute to own interlocking between sealer and dentine[5]. Therefore, the flow of endodontic sealers may be evaluated by the ISO 6876/2012[6] standard, which recommend that the materials have a minimum of 17 mm of flow. However, the main limitation of this conventional test is its incapability to evaluate also the filling ability of the materials[7].

A previous study[7] proposed the micro-computed tomography (micro-CT) as a reliable method to assess the flow and filling of root-end filling materials using a single test, in a 3D way. The authors used glass plates in a similar manner to the conventional ISO test, and observed an absence of correlation between the flow and filling properties. Up to now, there is no study applying this method to evaluate root canal sealers.

Endodontic sealers are divided according to their main components[8], and their properties are directly related to their composition[9]. AH Plus (Dentsply DeTrey, Konstanz, Germany) is an epoxy resin–based root canal sealer that is considered the gold standard for physical properties[10]. Fill Canal (Technew Com. e Ind. Ltda., Rio de Janeiro, RJ, Brazil) is a zinc oxide and eugenol-based sealer that is routinely used in the Brazilian dental practice[11]. Sealapex (SybronEndo, Glendona, CA, USA) is a root canal sealer based on calcium hydroxide[12]. Previous studies showed flow in accordance with the ISO 6876 for sealers based on calcium hydroxide, epoxy resin and zinc oxide and eugenol[12,13]. However, the comparison between flow and filling of these sealers was not assessed yet.

Based on this gap in the literature, the aim of this study was to evaluate the flow and filling ability of root canal sealers based on different components by using conventional tests and micro-CT. The null hypotheses were that there is no difference between the sealers, and the properties of flow and filling ability are associated.

MATERIAL AND METHOD

The endodontic sealers used in this study, their respective manufacturers, and compositions are described in Table 1.

Table 1. Endodontic materials, their manufacturers, and composition

Material	Manufacturer	Composition
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.
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.
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

Flow (mm and mm²)

The flow test was performed based on ISO 6876:2012 standard[6] (Figure 1). After mixing of the sealers, 0.05 mL of the material was placed in the center of a glass plate using a graduated disposable syringe (n = 10). Then, another glass plate (20 g) and a metal weight (100 g) were placed over the sealer, and kept for 10 minutes. So, the diameters of the sealer disks were measured using a digital caliper. The materials were photographed on the plate with a millimeter ruler for evaluation of the flow in area (mm²), according to Tanomaru-Filho et al.[14] The flow area of the sealers was obtained using Image Tool 3.0 software (UTHSCSA, San Antonio, TX, USA).

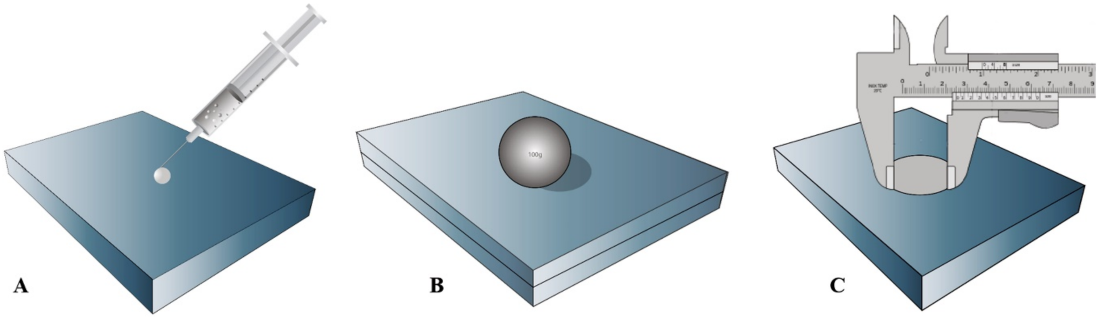

Figure 1. Schematic figure representing the flow assessment according to ISO 6876/2012. (A) 0.05 mL of the material was placed in the center of the glass plate by a graduated syringe; (B) Another glass plate and a metal load were placed over the sealer; (C) The diameters of the sealer disks were measured using a digital caliper.

Flow and Filling Ability Evaluated by Micro-CT

A glass plate was manufactured with a central cavity (1 × 1 × 2 mm) (length, width, and height) and grooves extending out horizontally and vertically to the 4 sides[7] (Figure 2A). Another dimension (1 × 1 × 1 mm - length, width, and height) was proposed for comparison. In a similar manner to the ISO 6876[6] test, an amount of 0.05 mL of each sealer was placed in the central cavity of the bottom glass plate, and another glass plate (20 g) and metal weight (100 g) were placed on the sealers and kept for 10 minutes (Figure 2B). The glass plates/sealer set was scanned with the SkyScan 1176 micro-CT system (SkyScan, Bruker, Kontich, Belgium) (Figure 2C). The micro-CT parameters were 9 µm voxel size, 90 kVp, 278 mA, 0.1 mm copper filter, and 360° scanning. The linear flow (mm) on each side of the grooves (horizontal and vertical) was analyzed. The filling in volume (mm³) of the sealers in the central area was determined as the central cavity filling. The filling in volume (mm³) of the materials in the lateral areas was determined up to 2 mm for each side of the central cavity. The images were reconstructed using NRecon software (V1.6.10.4; Bruker). The flow into the grooves besides the central and lateral fillings were calculated using the CTAn software (V1.15.4.0; Bruker).

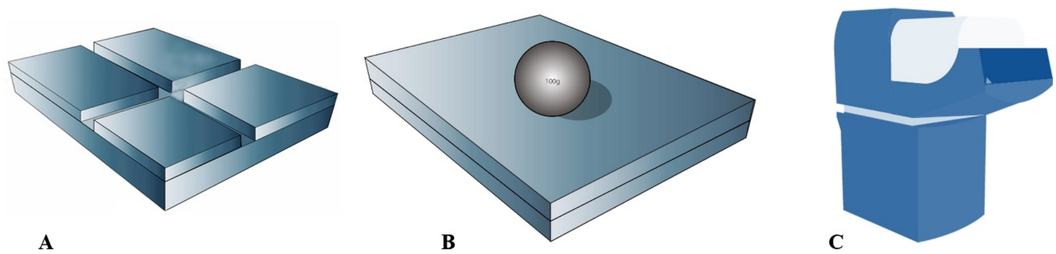

Figure 2. Schematic figure representing the flow and filling assessments by using micro-CT. (A) Glass plates were manufactured with a central cavity and grooves extending out horizontally and vertically; (B) Another glass plate and a metal load were placed after delivery 0.05 mL of the sealer in the central cavity of the bottom glass plate; (C) The glass plates/sealer set was scanned by micro-CT.

Statistical Analysis

The normality of the data was tested using the Kolmogorov-Smirnov test. Statistical analysis was performed with ANOVA and Tukey parametric tests. The level of significance was set at $p<0.05$.

RESULT

Flow (mm and mm^2)

All the sealers evaluated had a flow above the minimum (17 mm) recommended by the ISO 6876/2012. Although AH Plus had the highest flow value, there was no statistically significant difference among the materials ($p>0.05$). The assessment in area also showed similarities between the sealers ($p>0.05$). The results are represented in Table 2.

Table 2. Mean and standard deviation of the results of flow (mm and mm^2) of root canal sealers

	AH Plus	Fill Canal	Sealapex
Flow ISO 6876 (mm)	21.39 (0.76)	20.50 (1.64)	20.15 (0.76)
Flow area (mm^2)	546.1 (49.15)	536.7 (87.33)	527.6 (69.53)

There was no statistically significant difference between the sealers ($p>0.05$).

Flow and Filling Ability Evaluated by Micro-CT

The results are showed in Table 3. Independently of the test model used, there was no statistically significant difference between the linear flow (mm), central and lateral fillings (mm^3) observed for AH Plus, Sealapex and Fill Canal ($p>0.05$). All the sealers had a central filling above 80% and a lateral filling above 75%, in both diameters of test models.

Table 3. Mean and standard deviation of the results of flow (mm) and filling (%) of endodontic materials evaluated in micro-CT using test models with different sizes (length, width, and height)

	AH Plus	Fill Canal	Sealapex
Linear Flow (mm)			
1 × 1 × 1 mm	13.46 (1.49)	11.49 (1.28)	11.65 (1.24)
1 × 1 × 2 mm	8.32 (0.97)	9.25 (0.48)	8.99 (0.37)
Central Filling (%)			
1 × 1 × 1 mm	84.67 (3.20)	82.50 (4.18)	82.33 (10.09)
1 × 1 × 2 mm	89.50 (9.61)	80.75 (4.68)	89.42 (6.44)
Lateral Filling (%)			
1 × 1 × 1 mm	84.25 (5.65)	76.17 (6.55)	77.63 (12.72)
1 × 1 × 2 mm	86.68 (11.38)	76.11 (7.17)	85.46 (1.046)

There was no statistically significant difference between the sealers and between the different test models ($p>0.05$).

DISCUSSION

This study evaluated the flow and filling properties of endodontics sealers based on different components to compare the difference between the materials, besides differences between the properties evaluated. Our null hypothesis was accepted since there was no difference between the sealers and the flow and filling properties were also similar.

An appropriate flow ability of the root canal sealers is a desired property in order to fill irregularities[15]. Moreover, for the endodontic sealers to exert their antimicrobial properties, a proper flow able their diffusion into the complex anatomy of the root canal, which is difficult to access and disinfect[16]. Therefore, the root canal sealers could aid in the elimination of the remaining microorganisms, increasing the success rates of the endodontic treatment[17]. In this study, as previously performed, two methods of analysis were used to conventional evaluation of the sealers flow[12]. We used the method preconized by the ISO 6876/2012, which record the diameter of the sealers after to put a glass plate and a metal weight on the material. After that, we evaluated all the area occupied by the sealers, as an additional analysis[14].

All the sealers evaluated in the current study showed flow in accordance with the ISO[6] standard, which recommends a minimum value of 17 mm. Zinc oxide and eugenol-based materials are widely used in Endodontics[11]. However, in the best of our knowledge, there is no study evaluating the flow property for Fill Canal. Nevertheless, many studies evaluated other sealers based on zinc oxide and eugenol, such as Endofill (Dentsply-Maillefer, Dentsply Indústria e Comércio Ltda., Petrópolis, RJ, Brazil). Marin-Bauza et al.[13] attributed the high flow of Endofill to the presence of hydrogenated resin on its formula. This component is also present in Fill Canal. Although the flow value of AH Plus was numerically higher than Sealapex and Fill Canal, the sealers had a statistically similar flow. The high flow of AH Plus was described in previous studies[12,13,15-18]. The greater concentration of epoxy resin in AH Plus is considered the reason for its high flow rate[18]. The flow of the materials is also affected by the size of their particles, whereas small particles allow greater flow ability[18]. Chang et al.[19] showed similar flow diameter for AH Plus and Sealapex, in accordance with our findings. However, the authors found that the complex viscosities of these sealers were significantly different. Therefore, they stated that the flow of root canal sealers evaluated using the conventional test provides limited information, leading to the search of new methodologies to complement this assessment.

Micro-CT is an innovative tool that provide high-resolution 3-dimensional evaluation of root canal fillings, allowing further exploration of precise internal structures of the sealers in a nondestructive way[20]. For this reason, micro-CT is considered as the gold standard for the filling analysis[21]. Based on this important characteristic to an accurate evaluation, Tanomaru-Filho et al.[7] proposed a new technique for assessing flow and filling of root-end filling materials using micro-CT imaging. The authors stated that the test model proposed showed proper and reproducible results and could improve flow analysis. Moreover, this previous study observed an absence of correlation between the flow and filling properties. When using this technique to evaluate root canal sealers, and adding another diameter of test model, our results did not show difference between the sealers and the test models. These results probably occurred since no difference was observed in the flow property of the materials evaluated.

Araujo et al.[21] used micro-CT to evaluate AH Plus, Sealapex and Endofill regarding their volume in the 1 mm apical third after obturation of the teeth. In this study, the lowest volume of sealer was observed in teeth filled with Sealapex and Endofill. The authors considered this fact as a better sealing ability for these materials and related this characteristic to the greater flow rate of these sealers. AH Plus was also related to a good filling ability[**], besides a low percentage of voids[***].

CONCLUSION

Based on the methodologies used and the results obtained, we could conclude that AH Plus, Sealapex and Fill Canal have adequate flow and filling capacity, suggesting their clinical application. Micro-CT can be used to assess the flow and filling of root canal sealers by means of a single test.

**Roizenblit RN, Soares FO, Lopes RT, Santos BC, Gusman H. Root canal filling quality of mandibular molars with EndoSequence BC and AH Plus sealers: a micro-CT study. Aust Endod J. 2019 Sep 26. In press. http://dx.doi.org/10.1111/aej.12373. PMid:31556201.

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CONFLICTS OF INTERESTS

The authors declare no conflicts of interest.

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3.5 Publicação 5*

Effect of immersion in distilled water or phosphate-buffered saline on the solubility, volumetric change and presence of voids of new calcium silicate-based root canal sealers

Abstract

Aim To assess the effect of immersion in distilled water or phosphate-buffered saline (PBS) on the solubility, volumetric change and presence of voids of calcium silicate-based root canal sealers (TotalFill BC, Sealer Plus BC, and Bio-C), in comparison with the gold standard epoxy resin-based sealer (AH Plus).

Methodology All properties were evaluated after immersion in distilled water or PBS. Solubility was determined by the percentage of mass loss, whereas volumetric change and presence of voids were evaluated by micro-computed tomography, after 7 days of immersion. The volumetric change and percentage of voids between the baseline (after setting) and the experimental period were calculated. Statistical analysis was performed using one-way ANOVA and Tukey or Student's t-tests ($\alpha = 0.05$).

Results The calcium silicate-based sealers had significantly greater solubility and volumetric loss than AH Plus, after immersion in distilled water or PBS ($P < 0.05$). Bio-C had the greatest solubility ($P < 0.05$), followed by TotalFill BC and Sealer Plus BC, which were similar ($P > 0.05$). Regarding the volumetric change, AH Plus had a volume increase, with values similar to those of distilled water and PBS ($P > 0.05$). TotalFill BC, Sealer Plus BC, and Bio-C had a similar volumetric change ($P > 0.05$). The calcium silicate-based materials had the greatest solubility and volume loss after immersion in distilled water ($P < 0.05$). There was no difference in the percentage of voids among the sealers, before and after immersion in distilled water or PBS ($P > 0.05$).

Conclusions TotalFill BC, Sealer Plus BC, and Bio-C had significantly greater solubility and volumetric loss than AH Plus. Although storage in PBS significantly reduced the solubility and volumetric change of calcium silicate-based sealers, their solubility remained above that recommend by ISO 6876. All the sealers evaluated had low and similar voids, even after immersion in distilled water or PBS.

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Introduction

The development of biocompatible materials able to induce mineralized tissue deposition has been one of the main objectives of Endodontics (Almeida et al. 2018). Root canal sealers based on calcium silicates were developed as materials that offer biocompatibility, bioactivity and non-genotoxicity (Donnermeyer et al. 2018). Premixed, ready-to-use calcium silicate-based endodontic sealers, such as EndoSequence (Brasseler, Savannah, GA, USA), and TotalFill BC Sealer (FKG Dentaire, La Chaux-de-Fonds, Switzerland), have already been studied (Zamparini et al. 2019). TotalFill BC has adequate physicochemical properties, but seems to be soluble (Poggio et al. 2017, Tanomaru-Filho et al. 2017, Colombo et al. 2018). Recently, the new calcium silicate-based Sealer Plus BC (MK Life, Porto Alegre, RS, Brazil) and Bio-C Sealer (Angelus, Londrina, PR, Brazil) were developed and introduced in the market. To date, only one study has been conducted evaluating the physicochemical properties of Sealer Plus BC (Mendes et al. 2018). The authors observed adequate physicochemical properties, but greater solubility than that recommended by ISO 6876:2012. Regarding Bio-C, there are no studies on its properties in the literature.

Root canal filling materials should have low solubility, in order to prevent dissolution by body fluids (Urban et al. 2018). Moreover, the existence of voids inside the sealers can affect the filling quality (Huang et al. 2017). Although calcium silicate-based sealers have suitable physical properties, they have been reported to have high solubility when immersed in distilled water (Jafari & Jafari 2017, Urban et al. 2018). However, the use of simulated body fluids for immersion provides greater similarity to clinical application (Grech et al. 2013). Immersion of calcium silicate-based sealers in phosphate buffered saline (PBS) led to surface precipitation of calcium hydroxyapatite in vitro, evidencing the bioactivity of these sealers (Donnermeyer et al. 2018). Nevertheless, information in the literature is still sparse, and further investigations are needed to evaluate the clinical relevance of bioactivity and solubility properties (Donnermeyer et al. 2018). In addition, the presence of voids in these newly developed bioactive sealers has not yet been clearly explored (Huang et al. 2017).

It is also important to consider that calcium silicate-based materials can have both solubility and fluid absorption at the same time (Viapiana et al. 2014). When solubility is evaluated volumetrically, based on digital image analysis by micro-computed tomography (micro-CT), solubility and dimensional changes can be evaluated by the same test (Silva et al. 2017). Although TotalFill BC had high solubility when evaluated according to conventional tests, this material had volumetric stability in the micro-CT assessment (Tanomaru-Filho et al. 2017).

Based on this relevant information regarding calcium silicate-based sealers, and considering that the three-dimensional distribution of voids is important for canal filling quality, the aim of this study was to assess the effect of immersion in distilled water or PBS on the solubility, volumetric change and presence of voids of calcium silicate-based root canal sealers (TotalFill BC, Sealer Plus BC, and Bio-C), in comparison with the gold standard epoxy resin-based sealer (AH Plus, DeTrey/Dentsply, Konstanz, Germany). The null hypotheses were that there is no difference among the different sealers placed in the same immersion solution, and no difference for the same sealer placed in different immersion solutions.

Materials and Methods

The endodontic sealers and their respective manufacturers, compositions and proportions are described in Table 1.

Solubility evaluation

Based on a previous study (Carvalho-Junior et al. 2007), circular plastic moulds 1.5 mm-thick and 7.75 mm in diameter (n = 6) were filled with the sealers. An impermeable nylon thread was placed inside the fresh material. The moulds were placed on a glass plate covered with cellophane film and filled; then, another glass plate also covered with cellophane film was placed on the moulds, after which the assembly was placed in an incubator at 37°C and relative humidity of 95%, for a period corresponding to three times that of their setting time. Since calcium silicate-based sealers require moisture for setting, they were evaluated by placing 2 pieces of wet cloth between the mould and the glass plates, as proposed by Tanomaru-Filho et al. (2017). The specimens were then removed from their moulds, and weighed on an analytical balance (Ohaus Adventurer, Model AR2140, São Bernardo do Campo, SP, Brazil). The samples were placed inside plastic flasks containing 7.5 mL of distilled water, and kept in an oven at 37°C for 7 days. The nylon thread kept the specimens suspended and immersed in distilled water during the trial period, without allowing the samples to come into contact with the walls of the containers. After this period, the specimens were placed in the desiccator until they attained final mass stability. The solubility (mass loss) was expressed as a percentage of the original mass. This methodology was repeated for immersion of the samples in PBS.

Volumetric change and presence of voids: micro-CT evaluation

For the purpose of standardization, the samples for the volumetric change and void assessments were made in the same size as those for the solubility test. Circular plastic moulds 1.5-mm thick and 7.75 mm in diameter (n = 6), filled with the sealers, were first placed between two glass plates covered with cellophane film, and then in an incubator at 37°C and 95% relative humidity, for a period of three times the duration of their setting time. Since the calcium silicate-based sealers require moisture for setting, they were assessed by placing 2 pieces of wet cloth between the mould and the glass plates, as proposed by Tanomaru-Filho et al. (2017). Next, the specimens were removed from their moulds, and scanned by micro-CT (SkyScan 1176; Bruker-MicroCT, Kontich, Belgium). After the first scanning, the materials were placed in closed plastic flasks containing 7.5 mL of distilled water, and kept in an oven at 37°C for 7 days. Since the nylon thread for this test was not incorporated into the mass of the material, the position of the sealers was inverted in the plastic flasks after 3.5 days. As a result, the surface of the materials remained in contact with the liquid for the same period of time. After 7 days, the samples were placed in a desiccator under vacuum for 24 h, and scanned again. The scanning parameters were: 80 kV voltage, 300 µA current, 18 µm pixel size, copper and aluminium (Cu + Al) filter, and 360° rotation. The reconstruction of the images was performed using NRecon software (V1.6.10.4; Bruker-MicroCT). The correction parameters for smoothing, beam hardening, and ring artefacts were defined for each sealer. The same parameters were used for the same sealer at the different periods. The reconstructed images were superimposed on the different periods using the Data Viewer software (V1.5.2.4; Bruker-MicroCT). The 3D images were used for quantitative analysis of the samples, and allowed the total volume (mm^3) and presence of voids (%) in the materials to be calculated by CTAn software (V1.15.4.0; Bruker-MicroCT). The volumetric change and percentage of voids between the baseline and the experimental period were calculated. This methodology was repeated for immersion of the samples in PBS.

Statistical analysis

All the data were analysed with the GraphPad Prism 7.00 (GraphPad Software, La Jolla, CA, USA) statistical software package. Data were submitted to one-way ANOVA and Tukey or Student's t-tests ($\alpha = 0.05$).

Results

Solubility

After immersion in distilled water, all the calcium silicate-based sealers had high solubility values. The values after PBS immersion were significantly lower than after water immersion ($P < 0.05$). However, both values were higher than the minimum required by ISO 6876. Bio-C had significantly greater solubility ($P < 0.05$), followed by TotalFill BC and Sealer Plus BC, which had similar values ($P > 0.05$). AH Plus had the lowest solubility in both distilled water and PBS ($P < 0.05$), with similar values for both solutions ($P > 0.05$), and complied with the ISO standards. The results are described in Table 2.

Volumetric change

The volumetric changes observed for the root canal sealers are shown in Table 3. Figure 1 shows 3D models that illustrate the different sealers before and after immersion in PBS. AH Plus had the lowest volumetric change, regardless of the immersion liquid ($P < 0.05$). In addition, AH Plus was the only material that had a volume gain, with similar values for distilled water and PBS ($P > 0.05$). TotalFill BC, Sealer Plus BC, and Bio-C were similar ($P > 0.05$), and had a significantly greater volume loss when immersed in distilled water ($P < 0.05$).

Presence of voids

The presence of voids observed in the root canal sealers are presented in Table 4 and Figure 2. AH Plus, TotalFill BC, Sealer Plus BC, and Bio-C were similar ($P > 0.05$). There was no difference in the percentage of voids for all the materials after immersion in distilled water and in PBS ($P > 0.05$).

Discussion

This study assessed the solubility, volumetric change, and presence of voids of new calcium silicate-based sealers, in comparison with AH Plus, after immersion in distilled water or PBS. The null hypotheses were rejected, since several results observed between the sealers and the immersion solution were different, and affected the properties of the calcium silicate-based materials.

Root canal filling materials should have low solubility, in order to prevent microbial and fluid leakage (Zarra et al. 2018). AH Plus was less soluble than the calcium silicate-based sealers. Moreover, Bio-C had higher values than TotalFill BC and Sealer Plus BC. AH Plus is an epoxy resin-based sealer commonly used as a comparative material, because of its adequate properties (Silva et al. 2017). These properties include low solubility, since epoxy resin has strong cross-links (Viapiana et al. 2014). On the other hand, calcium silicate-based materials are more hydrophilic, and, therefore, more soluble (Siboni et al. 2017).

The current study evaluated the solubility of sealers based on a previous study that proposed smaller sample dimensions without affecting the accuracy of the test (Carvalho-Junior et al. 2007). Although the present study evaluated a different type of sealers, this methodology has been used to assess endodontic materials with various compositions, including calcium silicate-based materials (Viapiana et al. 2014, Tanomaru-Filho et al. 2017, Torres et al. 2018). This conventional test for solubility assessment is based on the measurement of the mass loss of the specimen before and after immersion in distilled water. However, the solubility of calcium silicate materials in distilled water does not express the real condition of the materials in vivo (Siboni et al. 2017). On the other hand, previous studies reported reduced solubility when calcium silicate-based materials were immersed in a simulated body fluid (Urban et al. 2018, Torres et al. 2018). Nevertheless, the present findings revealed that even after immersion in PBS, solubility remained above the minimum level recommended by ISO 6876.

Another limitation of conventional solubility tests is that specimens of materials with water uptake capacity may absorb water from the environment (Viapiana et al. 2014). Although water sorption is not evaluated using the volumetric change test, the comparison between the initial and final volumes in the digital image analysis allows the evaluation of material properties in a more clinical situation (Silva et al. 2017). According to this methodology, solubility and dimensional changes can be evaluated by the same experiment (Silva et al. 2017). Moreover, micro-CT allows longitudinal follow-up of the samples (Neves et al. 2019). This is the first study to report volumetric change values of endodontic materials after immersion in distilled water or PBS.

The present study showed that calcium silicate-based sealers had greater solubility than AH Plus. Moreover, calcium silicate-based sealers were associated with volume loss while AH Plus had volume gain after immersion in distilled water or PBS. For the purpose of standardization of the solubility test, the samples were stored in a desiccator for 24 hours after 7 days of immersion, before the final scanning process. This means that the reduction in the original weight or volume obtained by both methods was also caused by evaporation of the free fluids during the final drying of the samples (Gandolfi et al. 2015). Moreover, desiccation has a significant effect on sorption, solubility and volumetric expansion of water-based endodontic materials, since the cements not submitted to pre-storage desiccation are subject to positive volumetric expansion after water immersion (Mustafa et al. 2018).

In addition to low solubility, endodontic materials should not have a high percentage of voids (Gandolfi et al. 2016), since presence of voids may increase post-treatment apical periodontitis (Başer Can et al. 2017). Hence, the current study also investigated the presence of voids using micro-CT. Micro-CT is a highly accurate, nondestructive tool for measuring voids (Kim et al. 2018, Huang et al. 2018). A low percentage of voids was detected for the materials. The results corroborate previous studies comparing AH Plus and calcium silicate-based sealers (Celikten et al. 2016, Wang et al. 2018, Kim et al. 2019). This finding can be correlated with the small particle size and high viscosity of these sealers (Celikten et al. 2016, Huang et al. 2017). Nevertheless, a limitation of the present study was the pixel size of 18 μm applied during micro-CT scanning. Although the image resolution allowed voids to be segmented and quantified, smaller pixel sizes improve image quality and detection of voids (Huang et al. 2017, 2018, Kim et al. 2018, Orhan et al. 2018).

The present study revealed that although TotalFill BC, Sealer Plus BC and Bio-C had low voids, their solubility remained above the minimum recommended by ISO 6876, even after immersion in PBS. Therefore, since high solubility can compromise the long-term outcomes of root canal treatment, based on the ISO standard these sealers should not be used clinically. Further studies should be performed to acquire greater knowledge of the performance of calcium silicate-based sealers.

Conclusion

Calcium silicate-based sealers had significantly greater solubility and volumetric loss than AH Plus. All the sealers evaluated had similar low percentages of voids, even after immersion in distilled water or PBS. Although storage in PBS significantly reduced the solubility and volumetric change of TotalFill BC, Sealer Plus BC, and Bio-C, their solubility remained above the minimum amount recommend by ISO 6876. Therefore, according to ISO standards, these sealers should not be used clinically.

Acknowledgment

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Conflict of Interest statement

The authors declare explicitly that there are no conflicts of interest in connection with this article.

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Tables

Table 1 - Sealers, manufacturers, compositions and proportions used

Sealer	Manufacturer	Composition	Proportion
AH Plus	Dentsply DeTrey GmbH, Konstanz, Germany	Bisphenol A/F epoxy resin, calcium tungstate, zirconium oxide, silica, iron oxide pigments dibenzyldiamine, aminoadamantane, silicone oil.	1 g : 1 g (Paste/Paste)
TotalFill BC	FKG Dentaire SA, La Chaux-de-Fonds, Switzerland	Zirconium oxide, calcium silicates, calcium phosphate monobasic, calcium hydroxide, filler and thickening agents.	Ready to use
Sealer Plus BC	MK Life, Porto Alegre, RS, Brazil	Zirconium oxide, tricalcium silicate, dicalcium silicate, calcium hydroxide, and propylene glycol.	Ready to use
Bio-C Sealer	Angelus, Londrina, PR, Brazil	Calcium silicates, calcium aluminate, calcium oxide, zirconium oxide, iron oxide, silicon dioxide and dispersing agent.	Ready to use

Table 2 - Solubility values observed in root canal sealers after storage in distilled water and PBS (mean and standard deviation)

Solubility (% mass loss)	AH Plus	TotalFill BC	Sealer Plus BC	Bio-C Sealer
Distilled water	-0.02 (0.07)^{A,c}	7.82 (0.95)^{A,b}	6.45 (1.36)^{A,b}	20.53 (1.91)^{A,a}
PBS	-0.01 (0.10)^{A,c}	5.24 (2.09)^{B,b}	3.51 (1.12)^{B,b}	17.37 (2.47)^{B,a}

Different uppercase letters in same column represent significant differences for same sealer after immersion in different solutions ($P < 0.05$). Different lowercase letters on same line represent significant differences between different sealers ($P < 0.05$).

Table 3 - Volumetric change values observed in root canal sealers after storage in distilled water and PBS (mean and standard deviation)

Volumetric change (%)	AH Plus	TotalFill BC	Sealer Plus BC	Bio-C Sealer
Distilled water	0.88 (0.02)^{A,b}	-1.71 (0.36)^{A,a}	-1.07 (0.22)^{A,a}	-1.34 (0.29)^{A,a}
PBS	0.77 (0.11)^{A,b}	-0.53 (0.23)^{B,a}	-0.54 (0.27)^{B,a}	-0.58 (0.11)^{B,a}

Different uppercase letters in same column represent significant differences for same sealer after immersion in different solutions ($P < 0.05$). Different lowercase letters on same line represent significant differences among different sealers ($P < 0.05$).

Table 4 - Voids values observed in root canal sealers after setting (initial) and after storage in distilled water and PBS (mean and standard deviation)

Voids (%)	AH Plus	TotalFill BC	Sealer Plus BC	Bio-C Sealer
Initial	0.79 (0.34)	0.93 (0.13)	0.75 (0.24)	0.81 (0.13)
Distilled water	0.91 (0.13)	1.01 (0.12)	0.68 (0.19)	1.01 (0.28)
PBS	0.67 (0.22)	0.89 (0.15)	0.68 (0.16)	1.01 (0.25)

There was no significant difference for same sealer after immersion in different solutions ($P > 0.05$). There was no significant difference among different sealers ($P > 0.05$).

Figures

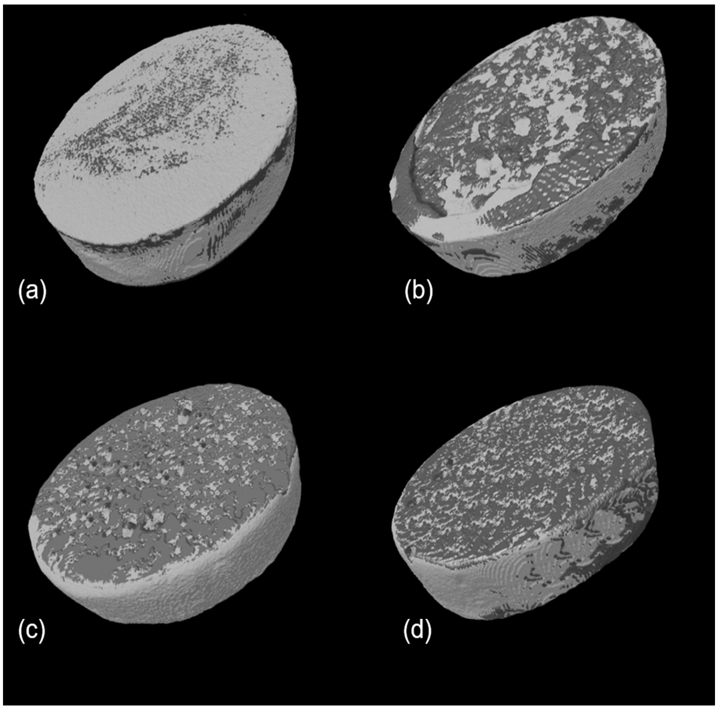

Figure 1 – 3D models using CTVol software, illustrating sealers (a: AH Plus; b: TotalFill BC; c: Sealer Plus BC; d: Bio-C Sealer), before (white) and after (grey) immersion in PBS during volumetric change evaluation.

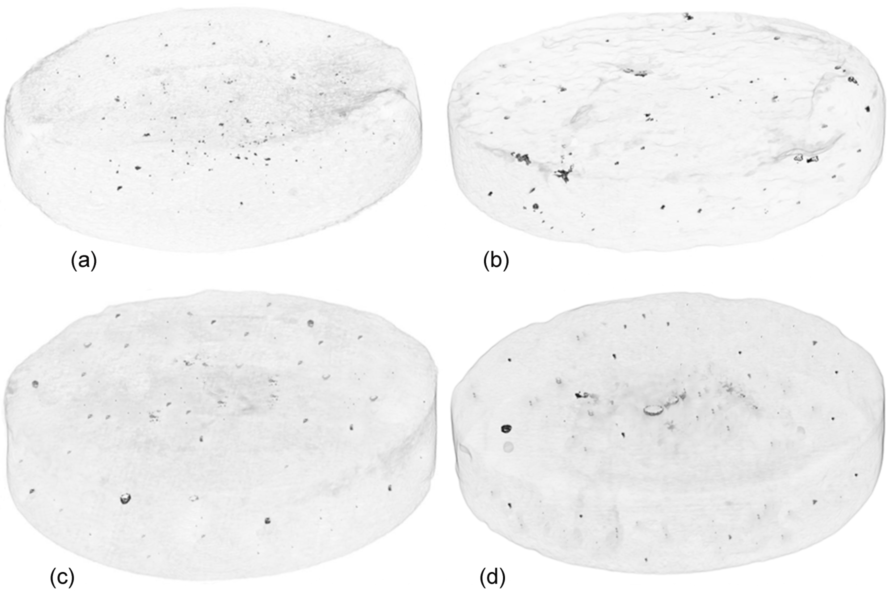

Figure 2 – 3D models using CTVox software illustrating sealers (a: AH Plus; b: TotalFill BC; c: Sealer Plus BC; d: Bio-C Sealer) regarding the presence of voids.

3.6 Publicação 6*

Micro-computed tomography high resolution evaluation of dimensional and morphological changes of 3 root-end filling materials in simulated physiological conditions

Abstract

The aim of this study was to evaluate volumetric and morphological stability of 3 root-end filling materials in addition to porosity and interface voids, using micro-computed tomography (μCT) in high resolution and a highly accurate approach for image analysis. Following root-end resection and apical preparation, two-rooted maxillary premolars were divided into three groups, according to the filling materials: White MTA Angelus, Biodentine, and IRM. Samples were scanned by μCT at 5 μm after the setting time and at time intervals of 7 and 30 days after immersion in phosphate-buffered saline (PBS). Volumetric and morphological changes besides material porosity and interface voids were evaluated by comparing initial values and those obtained after immersion. Data were analyzed statistically, using ANOVA and t-tests ($\alpha = 0.05$). All materials showed volumetric stability. Regarding the morphological changes, Biodentine had a significant thickness reduction after storage in PBS when compared with MTA. Biodentine also showed an increase in porosity, as well as in percentage and thickness of voids after 30 days of immersion. In conclusion, μCT in high resolution and an accurate image analysis approach may be used to evaluate morphological changes of endodontic materials. Although Biodentine showed suitable adaptability and lower values of porosity than MTA, after PBS immersion there was a dimensional reduction of this material, besides an increase in porosity and interface voids.

Keywords: Biomaterials, calcium silicate, endodontics, physical properties, x-ray microtomography.

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1 Introduction

Root repair materials should have appropriate physicochemical properties. Low values of solubility, dimensional changes and porosity are between these desirable characteristics. Solubility is an important property, because the dissolution of materials may allow leakage, leading to treatment failure [1]. Dimensional stability is also essential for endodontic materials, because shrinkage may harm the sealing ability [2]. Moreover, the porosity and microstructural defects of a material may reduce its hardness and strength. This combination could result in failures by means of formation and subsequent propagation of micro-cracks in the materials [3].

New methodologies, using micro-computed tomography (µCT), are used for evaluating physicochemical properties and associating volumetric change with solubility and dimensional change in endodontic materials [1]. µCT may be also used to determine the porosity, distribution, and size of pores within root-end filling materials [4, 5]. In addition, evaluating the interface between the dentin wall and endodontic cements by µCT make it possible to analyze the sealing ability of each material by the percentage of gaps and voids in this region [5, 6]. High resolution images provided by a small pixel size may have significant effect on the obtained results when evaluating porosity and other micro-structural features [7].

Calcium silicate–based repair cements are widely used in endodontics [8]. Mineral trioxide aggregate (MTA) is a dental material used for vital pulp therapy, internal/external resorption, perforation repair, and root-end filling [8]. However, MTA present some disadvantages as its consistency and long time required for setting [9].

To overcome the drawbacks of MTA, a range of bioactive endodontic cements has been developed to release calcium ions and form an interfacial layer between the cement and the dentin wall, which can be evaluated in a fluid environment such as phosphate-buffered saline (PBS) solution [10]. However, the bonding mechanism of this hydraulic cements at the material/dentine interface was not very well investigated [11]. Biodentine (Septodont, Saint Maur des Fosses, France) is a calcium silicate–based biomaterial and has indications similar to those of MTA [9]. With regard to the physicochemical properties, it has been suggested that Biodentine has a better consistency [9] but with higher solubility than MTA [4]. Intermediate restorative material (IRM. Dentsply DeTrey, Konstanz, Germany) is a reinforced zinc oxide-eugenol composition used as a root-end filling cement that could be used as a reference material in comparison with the new calcium silicate materials [12]. IRM has reported success rates similar to MTA [13].

Since the reports in the literature are still controversial, more researches are required to evaluate the difference between the root-end filling cements in order to make an informed decision to select the most effective material for clinical practice [13]. Moreover, there is no complete characterization of MTA, Biodentine and IRM changes after different periods of immersion in phosphate buffered saline using µCT in high resolution. Therefore, the aim of the present study was to evaluate volumetric and morphological stability, porosity and voids of Biodentine, MTA, and IRM before and after immersion in PBS between the experimental time intervals of 7 and 30 days using high-resolution µCT besides a highly accurate approach for image analysis.

2 Materials and Methods

2.1 Specimen preparation

Twelve extracted two-rooted maxillary premolars were used. Absence of anomalies in the root canal system was confirmed radiographically. This study was approved by the Local Research Ethical Committee (#CAAE: 9779617.5.0000.5416).

Root end resections were performed at 90° to the long axis of the root, ∼3 mm from the apex, using a tapered diamond drill 3080 FG–KG (KG Sorensen, Cotia, SP, Brazil) with a high-speed hand-piece under constant water spray irrigation. After apicectomy, 3 mm–deep cavities were prepared using an ultrasonic retrotip T1F-R (CVD-Vale, São José dos Campos, SP, Brazil) associated with a piezoelectric ultrasonic device (Ultrawave XS, Ultradent, South Jordan, Utah) appliance at a medium-power setting (50%) with distilled water irrigation.

Teeth were randomly divided into 3 groups of 8 roots with retrograde preparations. The cavities were filled with White MTA-Angelus (MTA; Angelus, Londrina, PR, Brazil) or Biodentine (Septodont, Saint Maur des Fosses, France) or IRM (Dentsply DeTrey, Konstanz, Germany), using a condenser kit (Ref.: 324501, numbers 2, 3, and 4; Golgran; São Caetano do Sul, SP, Brazil), by a single operator who was previously trained and calibrated. Materials were mixed according to the manufacturers' instructions. Samples were kept in an oven at 37 °C and 95% humidity for three times the duration of their setting time.

2.2 Image acquisition and export

All samples were scanned with the SkyScan 1272 μCT system (SkyScan, Bruker-microCT, Kontich, Belgium). The μCT parameters were 5 μm voxel size, 100 kVp, 100 μA, and 0.11 mm copper filter. After the scanning process, samples were kept immersed in PBS (1x) and stored in an oven at 37 °C between the experimental time intervals of 7 and 30 days. After these periods, new scans were performed using the same parameters. Therefore, the samples were scanned at day 1 (D1), day 7 (D7), and day 30 (D30). Data sets were reconstructed using NRecon software (V1.6.10.4; Bruker-MicroCT, Kontich, Belgium). The correction parameters were 4 for smoothing, 25% for beam hardening, and 5 for ring artifacts. All datasets were exported using the DICOM file format with an isotropic voxel size of 5 μm.

2.3 Image processing and segmentation

In a first step, images for each sample at D7 and D30 were spatially aligned with the reference image at D1, applying rigid transformation and maximization of mutual information as a registration metric [14] (Amira, FEI Visualization Sciences Group). All registered data sets were then imported for segmentation into a dedicated tool developed in MeVisLab (MeVis Research, Bremen, Germany) and validated for accurate tooth/root canal space segmentation [15]. Briefly, the segmentation tool applies semi-interactive livewire boundary extraction [16] to create a set of orthogonal contours, followed by a variational interpolation algorithm that reconstructs the surface of an object with energy-minimizing, smooth, and implicit functions [17].

2.4 Volumetric and morphological surface comparison

After segmentation, 3D triangular surfaces of the root-end filling materials at D1, D7, and D30 were constructed and the volumes were recorded. Morphological analyses were performed in 3-matic (Materialise, Leuven, Belgium). The material thickness at D1, D7, and D30 was measured to record the mean thickness. A signed, point-based, part comparison analysis for the three-dimensional (3D) surface of the fillings at different time points was performed to evaluate surface changes. This was performed by calculating the Euclidean distance between each point on the surface of the filling at D1 and its corresponding points at D7 and D30 and expressed as a color-coded map. The root mean square (RMS) of the calculated distances between different points on the surface of each sample (at D7 and D30) was then recorded.

In addition, porosity of the filling materials was analyzed from the segmented voxel images. Black top-hat transform was applied for pore segmentation, and then the total porosity was calculated as a percentage of the overall volume (Amira, FEI Visualization Sciences Group).

2.5 Interface analysis

Differences in the percentages of voids at the interface between the dentin surface of the root canal walls and the filling materials were evaluated based on the method described by Gandolfi et al. [6]. The 3D distribution of the interface voids in a predefined volume of interest (VOI) was calculated at D1, D7, and D30. Each 3D VOI was a 40-micron-thick interface volume consisting of the superficial 20 microns of the canal wall dentine and the first 20 microns of the filling material. Thus, voids with a size from 5 μm inside the VOI were detected by the threshold gray level. 3D models of the voids were created using CTAn software (V1.15.4.0; Bruker-MicroCT, Kontich, Belgium) and exported to 3-matic to analyze the voids' thickness.

2.6 Statistical analysis

All data were analyzed with the GraphPad Prism 7.00 (GraphPad Software, La Jolla, CA, USA) statistical software package. Data were submitted to normality test, and One-way ANOVA was used to test for statistical differences between different measurements within the same material (different time points) and between different filling materials. T-testing was used for part comparison analysis of the changes at two time points, D7:D1 vs. D30:D1 ($p < 0.05$).

3 Results

3.1 Volumetric and morphological changes

Volumetric and morphological changes observed for the materials are presented in Table 1, Figure 1, and Figure 2. For total volume, MTA, Biodentine and IRM were stable over time, with no statistical differences ($p > 0.05$). For morphological changes, MTA and IRM had stability after immersion ($p > 0.05$). Biodentine showed positive values at the apical part after immersion (Figs. 2d, 2e, and 2f). However, this material showed an overall reduction of its thickness at D30 ($p < 0.05$) (Fig. 1). These morphological differences were statistically significant between Biodentine and MTA at D7 in the analysis of morphological surface and at D7 and D30 in the analysis of the mean thickness ($p < 0.05$) (Table 1 and Fig. 1).

MTA and IRM maintained their porosity after immersion with similar values ($p > 0.05$) (Table 1). Although Biodentine showed an increase in its porosity after immersion ($p < 0.05$), this material presented lower values than MTA at all periods ($p < 0.05$).

3.2 Interface characterization

Figure 3 shows the interface evaluation of the cements. MTA and IRM kept their values of percentage and thickness of voids after immersion ($p > 0.05$). Biodentine showed an increase in the percentage of voids at D7 and D30, and an increase in void thickness at D30 ($p < 0.05$) in the intragroup evaluation. However, there was no difference between the filling materials in the percentage of voids at all periods ($p > 0.05$).

4 Discussion

Considering that root-end filling materials are expected to be in contact with body fluids [8, 18], the present study evaluated three materials over time in a simulated clinical condition. The results observed showed that the materials are volumetrically stable. However, Biodentine showed surface changes in the morphological analysis. This difference can be explained because the first measurement was performed for total volume of the materials in 3D, while the morphological changes were evaluated by means of a comparison for the material in the different periods. This test enables the comparison of initial and final images from each time period. From each comparison, raw coordinate data were generated representing between each individual point on the images [19]. Up to now, there are no studies in the specific literature using this method to evaluate root-end filling materials. Thus, the use of this approach for comparing new and reference materials could allow a detailed evaluation in order to assist the choice of the best material for clinical application.

According to the results obtained in the current study, Biodentine showed positive values in the apical part of the root, which can be observed in the color map of Figure 2, but a decrease in its overall thickness (Fig. 1). This thickness reduction may be related to the high solubility of Biodentine [18, 4, 20], which could be explained by a high dissolution of ions. Although this property may increase the solubility of the material, it may lead to deposition of calcium phosphate on the surface [20], which may be an advantage of this material from a biological perspective [21]. Moreover, the bioactive potential of cements based on calcium silicates seems to be a consequence of the solubility of these materials even after setting [22]. In this context, it was shown that the solubility of Biodentine in simulated body fluids was significantly lower than in distilled water [4, 12, 23], indicating the deposition of substances on the material and the bioactivity of this cement [23].

A recent study [24] comparing the wettability of MTA and Biodentine showed that the surface of MTA remained relatively unchanged after immersion in a simulated body fluid, and Biodentine presented changes, probably due its bioactivity and precipitation of crystals on its surfaces. Ashofteh Yazdi et al. [25] also detected a peak of Ca(OH)2 in Biodentine specimens exposed to PBS. Although this bioactivity cannot be analyzed by micro-CT, our 3D models showed a volume increase at Biodentine's surface (Fig. 2) and this possible deposition after superimposition of the images between the baseline and the time periods of immersion in PBS (Fig. 4). In spite of MTA is also considered a bioactive material, it was not feasible to observe in our 3D models this property as clearly as in Biodentine. This probably occurs due to the greater diffusion of ions in Biodentine, besides the crystallization of this material to resemble a lotus flower blossom and the precipitates found on MTA to be more peltiform [23].

IRM has been used for several decades [12] with success rates similar to MTA [13]. The present study showed a reduction of volume and thickness for IRM, mainly after 30 days of immersion. However, this reduction was not statistically significant. Our findings are in line with previous studies showing low solubility for this material [21] because eugenol is not water-miscible [12].

The properties of solubility and porosity may be associated [4]. Therefore, we can assume that the increase in Biodentine porosity after immersion occurred as a function of the material solubility. Despite this, our study observed a lower porosity for Biodentine in the initial period, which remained lower than MTA and similar to IRM after 7 and 30 days. These results are in agreement with a previous study, which observed lower porosity for Biodentine when compared to MTA [4]. The porosity observed for MTA may be associated with the presence of bismuth oxide as a radiopacifier, which can increase the relative porosity of calcium silicate-based cements [26]. Biodentine presents zirconium oxide as a radiopacifier that has a smaller particle size and higher filling ability for the spaces occupied by calcium [27]. These findings could justify the lower porosity of Biodentine when compared to MTA. IRM showed porosity values similar to Biodentine, in agreement with a previous study [28]. However, this is the first study to evaluate the porosity of IRM by using µCT. Besides that, the high resolution used in our study allowed a reliable detection of small porous inside the materials. This is important for the evaluation of endodontic materials, because higher resolution allows observation of micropores or very thin structures [7].

Low porosity and solubility for endodontic cements are desirable to provide a long-term seal and avoid leakage [24, 28]. The current study showed marginal adaptation for MTA, Biodentine, and IRM, with no differences between the percentage and diameter of voids at baseline. This adaptation of MTA was seen in a previous study [29], suggesting that the volume of pores inside MTA is a small fraction that does not affect the marginal adaptation [5]. Küçükkaya et al. [30] evaluated the filling quality of MTA and Biodentine placed with hand condensation or indirect ultrasonic activation, and observed similar obturation percentage volume of filling materials for Biodentine and MTA, in agreement with the present study.

An important limitation of the current study is that results from laboratory studies must be interpreted and extrapolated to clinical situations with caution [2]. Moreover, a recently published systematic review [31] stated that there is a deficiency in the development of standardized methods to evaluate the adaptation of root-end filling materials in ex vivo studies, including unit of gap measurement and magnification amount during analysis. Our study used µCT in order to investigate the interface between root canal walls and filling materials at a micrometer scale [32], since this tool shows superior features for sealing efficiency assessment [5]. µCT allows an accurate 3D analysis of the internal structure of the samples and the quantification of voids and gaps without the need to section the roots and create artifacts [33]. Furthermore, the high-resolution images provided nondestructively by µCT at 5 µm in the current study allowed also a qualitative analysis of the materials behavior over time, as observed in our 3D models.

The stability of the interface of biomaterial and dentine over time has a clinical significance in order to avoid the communication between possible residual bacteria and periapical tissue [34]. During the intragroup evaluation, we could observe an increase in the percentage of voids for Biodentine after immersion in PBS, besides the increase in the mean of the thickness of these voids after 30 days. Camilleri et al. [28] assessed the root dentin to material interface of Biodentine and IRM stored in Hank's balanced salt solution (HBSS), and observed that for IRM there were no gaps at the root dentin–cement interface, whereas Biodentine showed a gap of approximately 2 μm. Kim et al. [34] compared ProRoot MTA and Biodentine regarding the occurrence of mineral deposition at the interface between dentine and the materials after immersion in a simulated body fluid. The authors concluded that Biodentine displayed bioactivity by producing an interfacial layer on the root canal dentine. However, its thickness was significantly lower than MTA. This finding can justify our results and indicate that the phosphate from the soaking solution cannot reach the material/dentine interface as much as it would contact the material's surface [34].

The current study evaluated only some of the properties that may influence the quality of the root-end filling. However, the results of basic research protocols can contribute to the conduction of in vivo and clinical studies [35]. Therefore, this high-resolution analysis over 30 days seems promising to support the choice of the most appropriate endodontic material. Further studies should help elucidate the clinical impacts of these physicochemical properties in a longer observation during intraoral functioning.

5 Conclusions

The tests performed using μCT in high resolution are complementary and demonstrate the physicochemical behavior of the materials over time. MTA and IRM showed dimensional and volumetric stability. Biodentine showed low values of porosity and suitable adaptability at baseline, whereas, after immersion in PBS, this material showed a decrease in its thickness, besides an increase in porosity and interface voids.

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Authorship declaration All authors have contributed significantly and they are all in agreement with the manuscript.

Conflict of interest The authors declare that they have no conflict of interest.

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Table

Table 1 Volumetric and morphological changes, porosity, and voids in the different time intervals (mean and standard deviation) observed in endodontic materials

	MTA	Biodentine	IRM
Total volume (mm³)			
D1	1.23 (0.19)^{Aa}	1.24 (0.12)^{Aa}	1.19 (0.24)^{Aa}
D7	1.24 (0.19)^{Aa}	1.24 (0.11)^{Aa}	1.18 (0.23)^{Aa}
D30	1.24 (0.20)^{Aa}	1.23 (0.11)^{Aa}	1.17 (0.23)^{Aa}
Morphological surface changes (RMS) (µm)			
D7	9.70 (6.00)^{Aa}	15.00 (5.00)^{Ab}	9.90 (5.60)^{Aab}
D30	10.00 (4.00)^{Aa}	21.00 (8.10)^{Bb}	15.00 (8.40)^{Aab}
Porosity (%)			
D1	8.93 (1.68)^{Aa}	5.73 (0.89)^{Ab}	8.51 (0.88)^{Aa}
D7	10.10 (2.56)^{Aa}	6.79 (0.89)^{Bb}	7.91 (1.56)^{Aab}
D30	11.35 (3.16)^{Aa}	6.45 (1.13)^{Bb}	7.98 (1.32)^{Aab}
Interface voids (%)			
D1	2.04 (1.44)^{Aa}	2.06 (1.47)^{Aa}	2.01 (1.06)^{Aa}
D7	2.29 (1.26)^{Aa}	2.82 (1.58)^{Ba}	2.49 (1.33)^{Aa}
D30	1.53 (1.25)^{Aa}	3.03 (1.84)^{Ba}	2.98 (1.53)^{Aa}
Interface voids thickness (µm)			
D1	7.11 (0.46)^{Aa}	7.56 (0.64)^{Aa}	7.04 (1.01)^{Aa}
D7	7.04 (0.71)^{Aa}	7.77 (0.63)^{Aa}	7.15 (0.91)^{Aa}
D30	7.04 (0.61)^{Aa}	8.52 (0.59)^{Bb}	7.50 (1.05)^{Aa}

^{AB}Different capital letters in the same column indicate statistically significant difference between the same materials in the different time intervals ($p < 0.05$).
^{ab}Different lowercase letters on the same row indicate statistically significant difference between the different materials ($p < 0.05$).

Figures

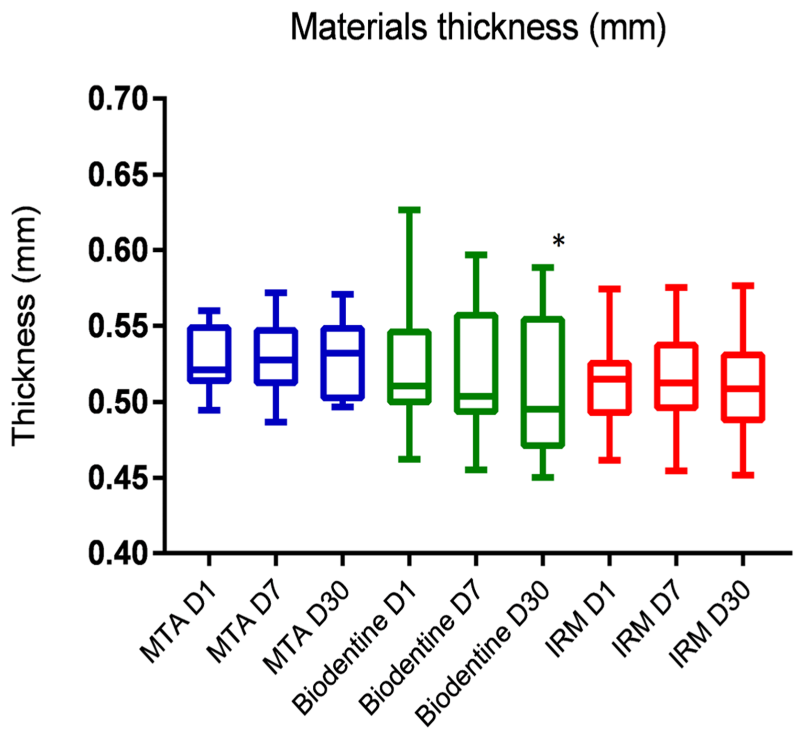

Fig. 1. Box plot graph representing the material's thickness (mm) at D1, D7, and D30. The asterisk indicates statistical difference.

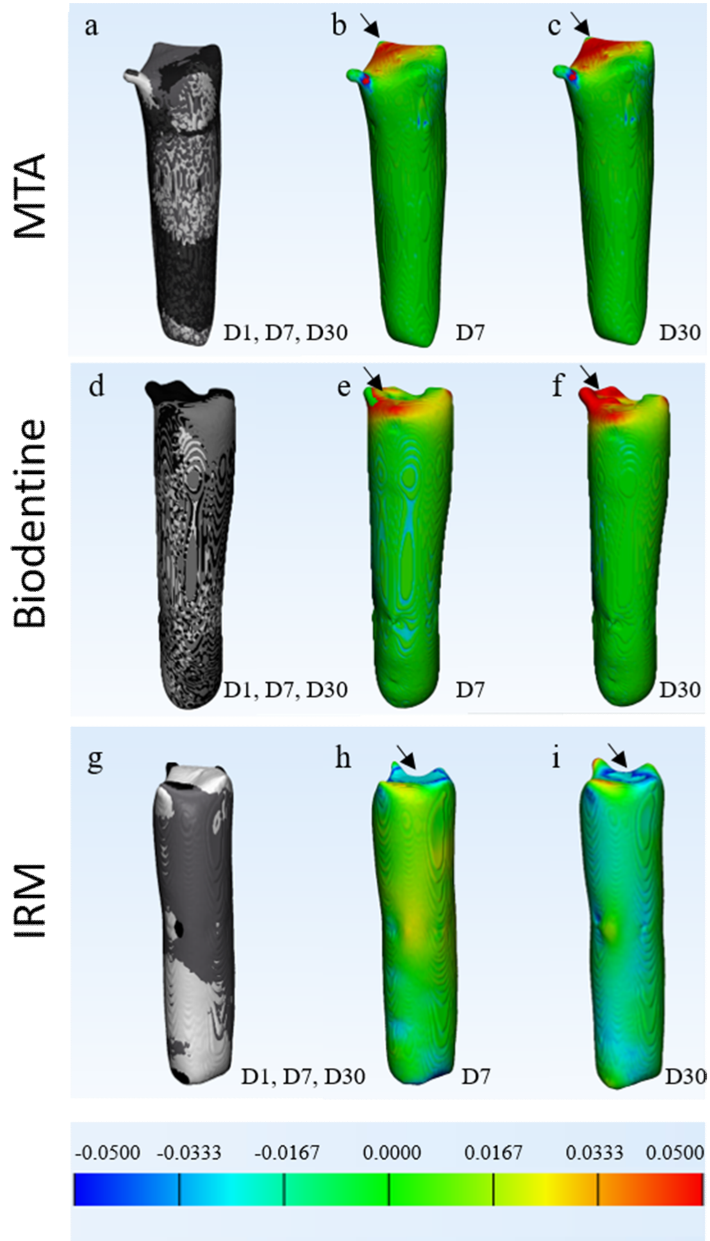

Fig. 2. 3D models, using 3-matic software showing the morphological surface changes of MTA (a), Biodentine (d), and IRM (g) after superimposition at D1 (white), D7 (gray), and D30 (black); morphological changes at D7 for MTA (b), Biodentine (e), and IRM (h); morphological changes at D30 for MTA (c), Biodentine (f), and IRM (i); The color map shows the values of the changes (mm). The arrows show the volume increase on MTA and Biodentine's surface.

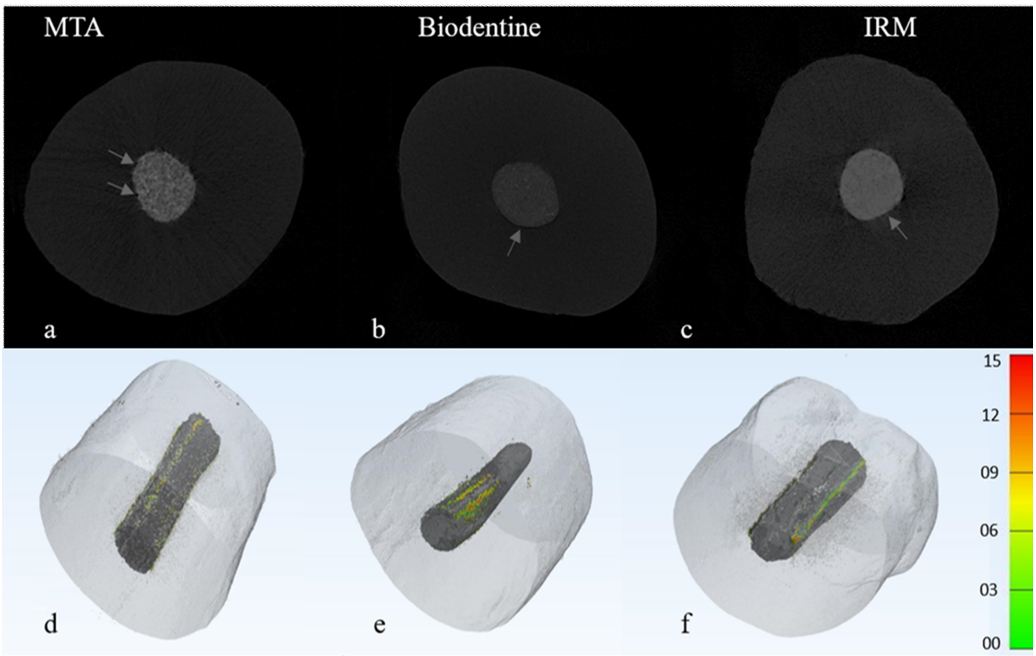

Fig. 3. Images of the interface voids and gaps during the assessment, using CTAn software for MTA (a), Biodentine (b), and IRM (c). The arrows show the interface voids/gaps observed for each material. 3D models, using 3-matic software showing the interface void thicknesses (µm) for MTA (d), Biodentine (e), and IRM (f).

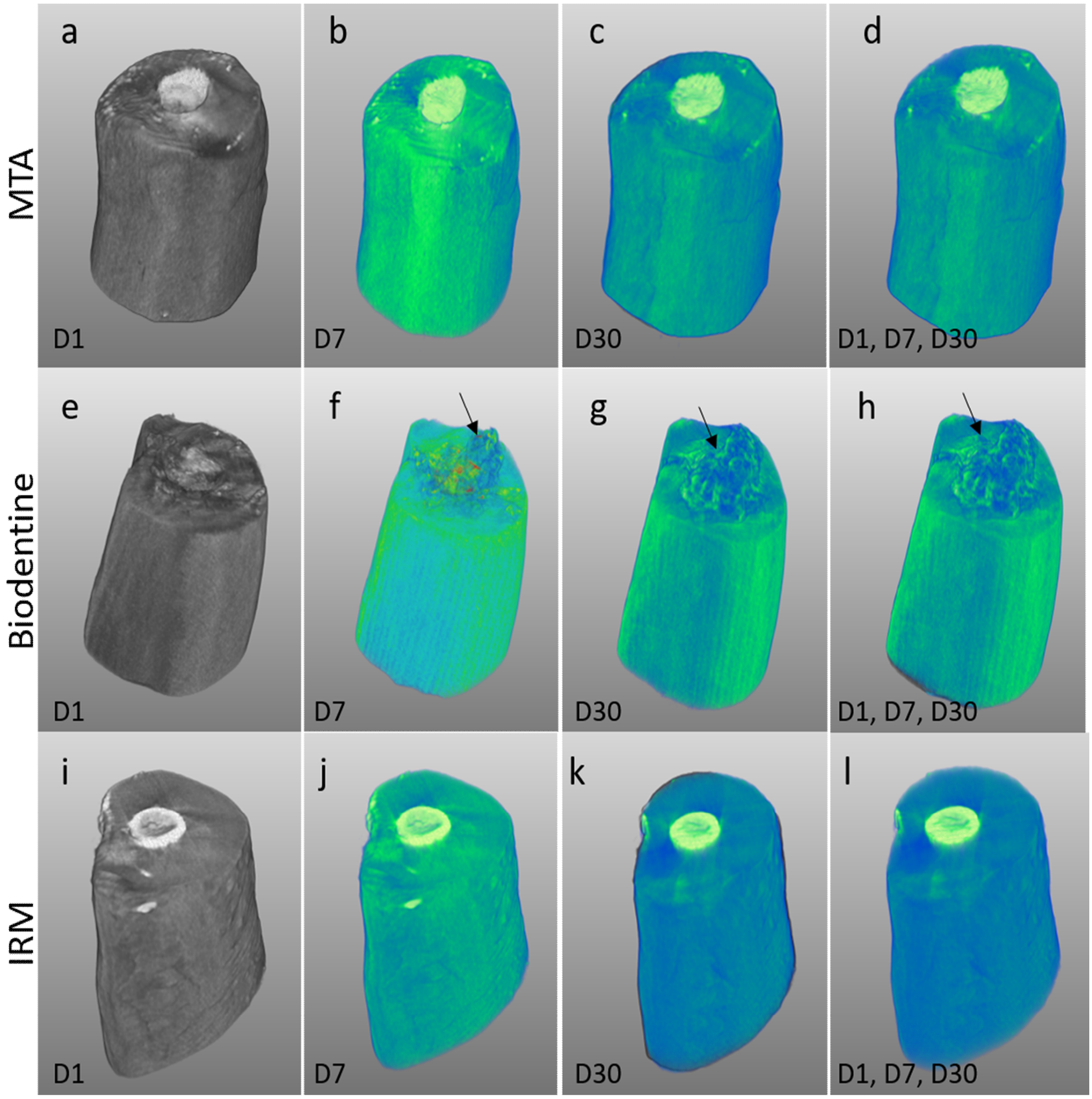

Fig. 4. 3D models, using Amira software representing MTA, Biodentine and IRM, respectively, at D1 (a, e, i), D7 (b, f, j), and D30 (c, g, k) and superimposition of D1, D7, and D30 (d, h, l). The arrows show the possible mineral deposition on Biodentine's surface.

3.7 Publicação 7*

How image-processing parameters can influence the assessment of dental materials by micro-CT

ABSTRACT

Purposes: To evaluate the influence of voxel size and different post-processing algorithms on dental materials analysis by micro-computed tomography (micro-CT).

Materials and Methods: Root-end cavities were prepared in extracted maxillary premolars, filled with mineral trioxide aggregate (MTA), Biodentine and Intermediate Restorative Material (IRM), and scanned using micro-CT. Volume and porosity of materials were evaluated using voxel sizes 5, 10, and 20 μm, and different software tools (post-processing algorithms), which were compared. CTAn or MeVisLab/Materialise 3-matic software packages were used to perform volume and morphological analysis, and CTAn or MeVisLab/Amira software, for porosity evaluation. Data were submitted to one-way ANOVA and Tukey's statistical tests ($P < 0.05$).

Results: Volume increased when voxel size increased, according to cement analysis using MeVisLab/Materialise 3-matic. CTAn showed an increase in volume for MTA and IRM at 20 μm. Pore values decreased when voxel sizes increased, with statistically significant difference for all materials, as evaluated using CTAn. MeVisLab/Amira showed a difference for MTA and IRM at 5 μm, and for Biodentine at 20 μm. Significant differences were observed among the software packages when Biodentine was assessed regarding volume and porosity at all voxel sizes.

Conclusion: There were some differences of volume and porosity according to voxel size, image-processing software, and materials' radiopacities. The consistent protocols are needed for the researches about evaluating dental material.

KEY WORDS: Dental materials; Image processing; Three-dimensional imaging; X-ray microtomography

* Artigo escrito nas normas do periódico *Imaging Science in Dentistry*, ao qual foi submetido e aceito para publicação. A política de copyright e autoarquivo de editores para esta revista está disponível no Anexo H.

Introduction

Dental research has revealed how important tridimensional (3D) imaging is in the clinical field and in researching different materials.[1] Microcomputed tomography (micro-CT) is a 3D, nondestructive imaging method considered the gold standard for in vitro applications,[2] since it provides more detailed information about smaller structures.[3] This 3D modality has often been used to assess cement volumes,[4,5] and determine obturation porosity.[6,7] However, evaluation of dental materials still poses a challenge in some images, since the currently available cements can generate artifacts, and thus decrease overall image quality.[2] Moreover, there is a lack of standardization in image processing parameters, which can influence the results of the research.

One of the steps that influences accurate analysis of micro-CT images is segmentation of the region of interest.[8] Several segmentation methods are available and may influence the outcomes differently.[9] Manual segmentation is a time-consuming task requiring an experienced operator, and providing low repeatability and reproducibility.[10] Conversely, semi-automatic and automatic methods are faster, and new, less operator-dependent mathematical processing algorithms are being developed to process large volumes of data.[11]

A fundamental part of the research after the image segmentation step is image analysis.[12] Morphometric indices can be derived from either a simple voxel-counting method or more advanced volume-rendering methods. Different manufacturers provide distinct software packages that can be used to compute these indices.[13]

The demands on image segmentation and analysis become even more stringent when the image quality is limited due to low spatial resolution and contrast, or to a great amount of noise.[14] In this challenging situation, a factor that may assist the computer (algorithm) to better define the region/structure boundaries is improved image resolution.[11,15-17] Although high resolution provides some advantages, such as greater precision,[18] the drawback is that it requires a greater amount of data, scanning time and analysis,[19] thus raising the cost and extending the rendering time of the research.

Imaging processing parameters for evaluating volume and porosity of dental materials by using micro-CT have still not been standardized. Consequently, studies are unable to assess the differences observed in imaging investigations using different spatial resolution, or software that perform these analyses.

Recommendations for imaging protocols are needed to reduce measurement error and scanning time.[20] Thus, the aim of this study was to evaluate the impact of voxel size and different post-processing algorithms on the precision of measurements regarding volume and porosity of dental materials.

Materials and Methods

Specimen preparation

After obtaining approval from the Institutional Ethics Committee (Registration number #CAAE: 9779617.5.0000.5416), twelve extracted two-rooted maxillary premolars without any anomalies in the root canal system were selected. Root-end resection was performed at 90° from the long axis of the root, ~3 mm from the apex. After apicectomy, 3.0-mm deep cavities were prepared using an Ultrawave device (Ultrawave XS, Ultradent, South Jordan, Utah) with ultrasonic retrotip T1F-R (CVD-Vale, São José dos Campos, SP, Brazil).

The teeth were divided randomly into 3 groups, and the cavities were filled with mineral trioxide aggregate (MTA) (Angelus, Londrina, PR, Brazil) or Biodentine (Septodont, Saint Maur des Fosses, France) or Intermediate Restorative Material (IRM) (Dentsply DeTrey, Konstanz, Germany) by a single, previously trained and calibrated operator. The materials were mixed according to the respective manufacturer's instructions. The samples were kept in an oven at 37°C and 95% humidity three times longer than their recommended setting time.

Image acquisition and reconstruction

The specimens were scanned by micro-CT (SkyScan 1272, Bruker, Kontich, Belgium) using the following acquisition parameters: 5 μm voxel size, 100 kVp, 100 μA, 0.11 mm copper filter, 4 frame average, and 180º rotation. Datasets were reconstructed using NRecon software (V1.6.10.4; Bruker, Kontich, Belgium). The correction parameters were 40% for smoothing, 25% for beam hardening, and 25% for ring artifacts. All the datasets were exported using the DICOM file format with an isotropic voxel size of 5 μm. Schematic illustration of specimen preparation and evaluation is represented in Figure 1.

Image segmentation and volume calculation

The filling materials were segmented and their total volume were recorded at 5 μm, 10 and 20 μm. Segmentation was performed using two different protocols: 1- CTAn (V1.15.4.0; Bruker, Kontich, Belgium) using adaptive thresholding, bearing in mind that segmentation can be improved by using local threshold values instead of a single global threshold value.[21] In this method, the examiner segments the images visually, and records the threshold values for each sample.[9] 2- MeVisLab (MeVis Research, Bremen, Germany) uses a semi-interactive livewire boundary extraction[10], which the user moves the mouse from a manually selected point, and a livewire boundary selects and wraps around the object of interest. Then new points are selected, until the boundary has been delimited and the area has been segmented.[22] This method creates a set of orthogonal contours, and then applies a variational interpolation algorithm that reconstructs the surface of the object[23,24], providing an interactive segmentation that combines examiner recognition with outperformance of a computer to achieve synergistic delineation.[22]

After segmentation using MeVisLab, the reconstructed 3D surfaces were exported to 3-matic (Materialise; Leuven, Belgium) for volume quantification and morphological analysis, by superimposition of the reconstructed 3D surfaces (5, 10 and 20 μm), to obtain a color-coded map showing the surface deviation of the segmented objects.

Materials porosity analysis

After segmentation by CTAn software, the porosity of each material was calculated using this same package. In comparison, after segmenting the materials using MeVisLab, the image was exported to Amira (Amira, FEI Visualization Sciences Group) to calculate porosity. The pores were segmented using top-hat transformation.

Statistical analysis

All the data were analyzed with the GraphPad Prism 7.00 statistical software package (GraphPad Software, La Jolla California USA). One-way ANOVA was used to evaluate the statistical differences between voxel sizes, and the differences in the volume and porosity measurements using several software packages at 5, 10, and 20 μm. The level of significance was set at $P < 0.05$.

Results

Materials volume

Table 1 presents the total volume measurements. MTA and IRM showed a statistically significant increase in volume only when analyzed at 20 μm by CTAn, whereas Biodentine had different results at all voxel sizes ($P < 0.05$). The same results were observed in the comparison between software packages; that is, the images for MTA and IRM were different only at 20 μm, whereas the values obtained for Biodentine were different at all voxel sizes according to the software programs ($P < 0.05$). The increase in volume was statistically significant for the three cements according to MeVisLab/Materialise 3-matic ($P < 0.05$) (Table 1). Figure 2 shows the 3D models of each material at 5, 10 and 20 μm, before and after superimposition using a color map that shows the increase in volume (mm) after the voxel size is increased.

Materials porosity

The results of porosity, considering all voxel sizes and software packages, are presented in Table 2. Unlike the volume analysis, the porosity of the materials decreased from 5 to 10 and to 20 μm resolutions (Table 2). Figure 3 shows 3D models representing the porosity of each material observed at 5, 10 and 20 μm, with a color map that shows the thickness (mm) of the pores. According to CTAn software, the porosity measured was found to be significantly different among the voxel sizes for MTA, IRM, and Biodentine ($P < 0.05$). According to MeVisLab/Amira software, the porosity values for MTA and IRM were different at 5 μm ($P < 0.05$), whereas the values for 10 and 20 μm were similar ($P > 0.05$). Biodentine at 20 μm showed different results ($P < 0.05$).

Regarding the software packages, the values obtained for MTA and IRM were similar using CTAn and MeVisLab/Amira ($P > 0.05$). In contrast, Biodentine showed significant differences independent of the voxel size ($P < 0.05$).

Discussion

Evaluation of volumetric and morphological stability and of porosity of dental materials may play an important role in determining the most appropriate material for each treatment, and thus improve the clinical success rate over time. Many physicochemical properties of dental materials are poorly investigated, and the data published in the literature are controversial.[25]

In recent years, there have been many innovative advances in high resolution imaging modalities and in image post-processing procedures.[26] High-resolution micro–CT imaging has been widely applied to evaluate dental materials using different approaches for image acquisition and assessment, and reporting of outcomes. However, this has led to a lack of consistency that makes it difficult to interpret results and to compare findings across different studies.[13] The current study shows that voxel size and post-processing algorithms influence the evaluation of volume and porosity of dental materials using micro-CT images.

Regarding the impact of voxel size on materials volume analyses, an increase in the voxel size was found to match the increase in volume, with an error of approximately 1 and 4%, using CTAn and MeVisLab/Materialise 3-matic software, that is, from 5 to 10 μm, respectively. At 20 μm, the error increased to 2% using CTAn, and to 13% using MeVisLab/Materialise 3-matic. Moreover, when Biodentine was used—a material with low radiopacity—[27] the difference between voxel sizes and software packages was more evident.

This can be attributed to the partial volume effect that occurs when neighboring voxels include multiple materials. During image acquisition, anatomical structures are discriminated based on their radiographic density. Therefore, voxels at the external surface of the sample may contain the densities of the two materials.[28-30] Although the parameters for image correction of smoothing, beam hardening and ring artifacts can be applied during reconstruction of the images, artifact reduction tools applied to micro-CT images could have no significant influence on objective image analysis.[31] Hence, lower spatial resolution associated with low contrast between the structures[32] may induce this kind of error, resulting in overestimation of some parameters, thickening of structures, and a greater volume percentage.[33]

Conversely, porosity assessment showed a decrease in values when the voxel size increased. A decrease of approximately 7% in porosity by increasing the voxel size from 5 to 10 μm was observed using CTAn, and 12% using MeVisLab/Amira.

When images with 20 µm were evaluated, a reduction in porosity of up to 26% was observed using CTAn and 24% using MeVisLab/Amira. This variation occurred because resolutions exceeding the dimensions of micropores, or very thin structures, preclude the visualization of small features.[3,34]

The fact that high porosity can be associated with a decrease in the flexural strength of dental materials[6] makes the evaluation of this property clinically valuable. Accordingly, high resolution can help the researcher better distinguish small pores and voids inside the cements.[35] In agreement with our results, other studies have demonstrated that cortical bone porosity analysis depends on voxel size[16] and also have suggested a voxel size of 11.2 µm as a cut off value for the evaluation of root canal filling voids.[36]

The differences observed in the current study serve to highlight the influence of the post-processing algorithms on analysis of dental materials by micro-CT.[37] Thresholding can be performed by simple global or by adaptive methods using CTAn software.[9] Our study used the adaptive method because it applies local threshold values instead of a single global method. As a result, segmentation could be improved to the extent that the threshold value of each voxel could be determined optimally within its neighborhood.[21] Segmentation using MeVisLab was performed by applying interactive livewire boundary extraction to select the region of interest from the surrounding area.[24] This is an interactive tool that provides efficient, accurate and reproducible boundary extraction.[10]

Image analysis is the next most critical step after segmentation. Different algorithms are implemented in order to compute several indices.[13] In performing 3D morphometric analysis using CTAn software, the marching cubes algorithm is used to calculate values for surfaces and volumes, and ensures more accurate measurements than simple pixel counting techniques. This algorithm is a traditional method preferred for its simplicity and efficiency.[38] Volume analysis using Materialise 3-matic was performed by importing an STL file after segmentation of the dental materials by using MeVisLab software. Since STL is a surface representation, a tetrahedral volume was generated from a triangle surface mesh to perform the analysis in 3-matic. This method allows the user to determine what parameters and settings are most important, depending on application and preference. The resulting algorithm produces an effective assessment.[39]

The segmented images acquired with MeVisLab were also used to perform porosity analysis by Amira. The black top-hat transform algorithm was applied in this software to perform pore segmentation. Afterwards, total porosity was calculated as a percentage of the overall volume. Black top-hat transformation is an image processing technique for extracting dark features on a variable background that offers more accurate estimates of volumes.[40]

Preferably, the smallest voxel size should be used for small structure scans, aimed at obtaining more accurate measurements.[18,21,41,42] Comparatively, higher resolution scans require longer acquisition times, because they must collect more projections and generate large datasets. Therefore, the tradeoff between voxel size and scan time should be carefully considered.[13] It is important to underscore that variations in the values obtained using different software packages were more common when larger voxel sizes were used. Nevertheless, although our results have shown that smaller voxel sizes would be advantageous, the use of larger voxel sizes should not be discouraged when applicable.

Conclusions

Micro-CT analysis of volume and porosity of dental materials showed a distinct outcome based on voxel size, post-processing algorithms, and materials' radiopacities. The similarities between the software-based outcomes and the reliability of the results increased due to the small voxel sizes and the radiopacity of the materials. Therefore, consistent protocols are critical to making inter-study comparisons of micro-CT results, and should be undertaken to ensure selection of the best indicated dental material.

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Tables

Table 1 - Volume values (mm³) using different voxel sizes and software packages (mean and standard deviation) to analyze the dental materials

	MeVisLab/Materialise 3-matic	CTAn
MTA		
5 µm	1.24 (0.19)*	1.23 (0.19)
10 µm	1.28 (0.20)*	1.24 (0.19)
20 µm	1.39 (0.21)*	1.25 (0.19)* †
Biodentine		
5 µm	1.24 (0.11)*	1.13 (0.11)* †
10 µm	1.30 (0.09)*	1.14 (0.11)* †
20 µm	1.38 (0.12)*	1.15 (0.11)* †
IRM		
5 µm	1.18 (0.23)*	1.18 (0.12)
10 µm	1.23 (0.23)*	1.20 (0.17)
20 µm	1.37 (0.23)*	1.22 (0.17)* †

*: Significant differences were detected among the voxel sizes ($P < 0.05$).
†: Significant differences were detected among the software packages ($P < 0.05$).

Table 2 - Porosity values (%) using different voxel sizes and software packages (mean and standard deviation) to analyze the dental materials

	MeVisLab/Amira	CTAn
MTA		
5 µm	10.1 (2.6)*	9.1 (1.3)*
10 µm	8.2 (2.0)	8.5 (1.4)*
20 µm	7.8 (1.8)	6.9 (1.7)*
Biodentine		
5 µm	6.2 (1.0)	5.0 (1.2)* †
10 µm	6.1 (1.1)	4.4 (1.2)* †
20 µm	5.2 (1.0)*	3.2 (1.0)* †
IRM		
5 µm	8.1 (1.3)*	7.8 (1.3)*
10 µm	6.8 (1.8)	7.4 (1.3)*
20 µm	5.3 (1.0)	6.4 (1.3)*

*: Significant differences were detected among the voxel sizes ($P < 0.05$).
†: Significant differences were detected among the software packages ($P < 0.05$).

Figures

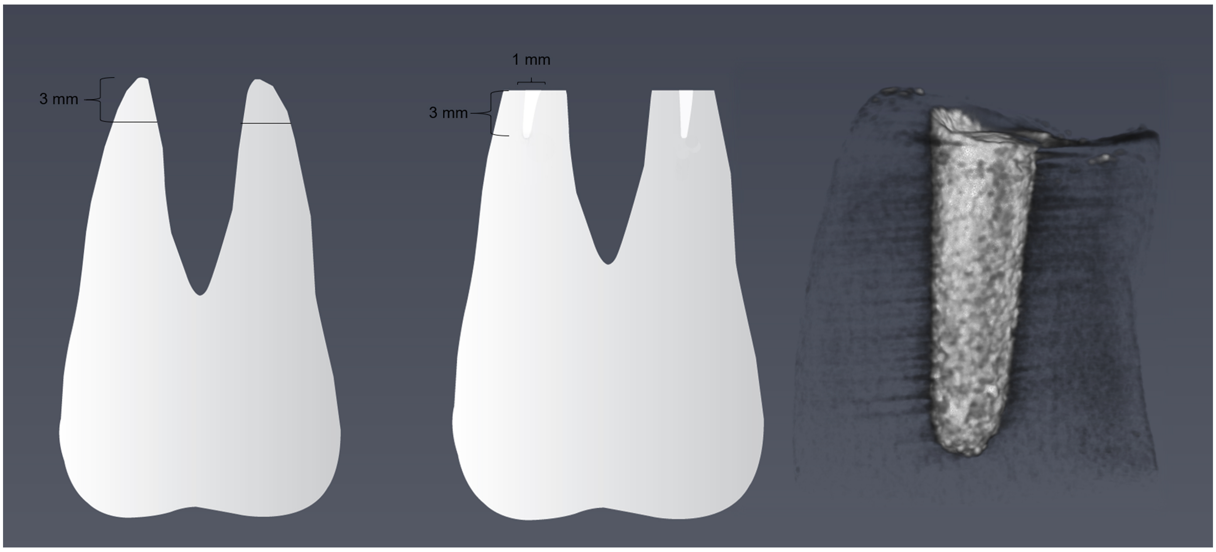

Fig. 1. Schematic figure representing tooth preparation and evaluation of materials. Root-end resection was performed at 90° from long axis of the root, ~3 mm from the apex. Thus, 3.0-mm deep cavities were prepared and filled with the root-end filling materials. The materials were assessed after micro-CT scanning and image reconstruction.

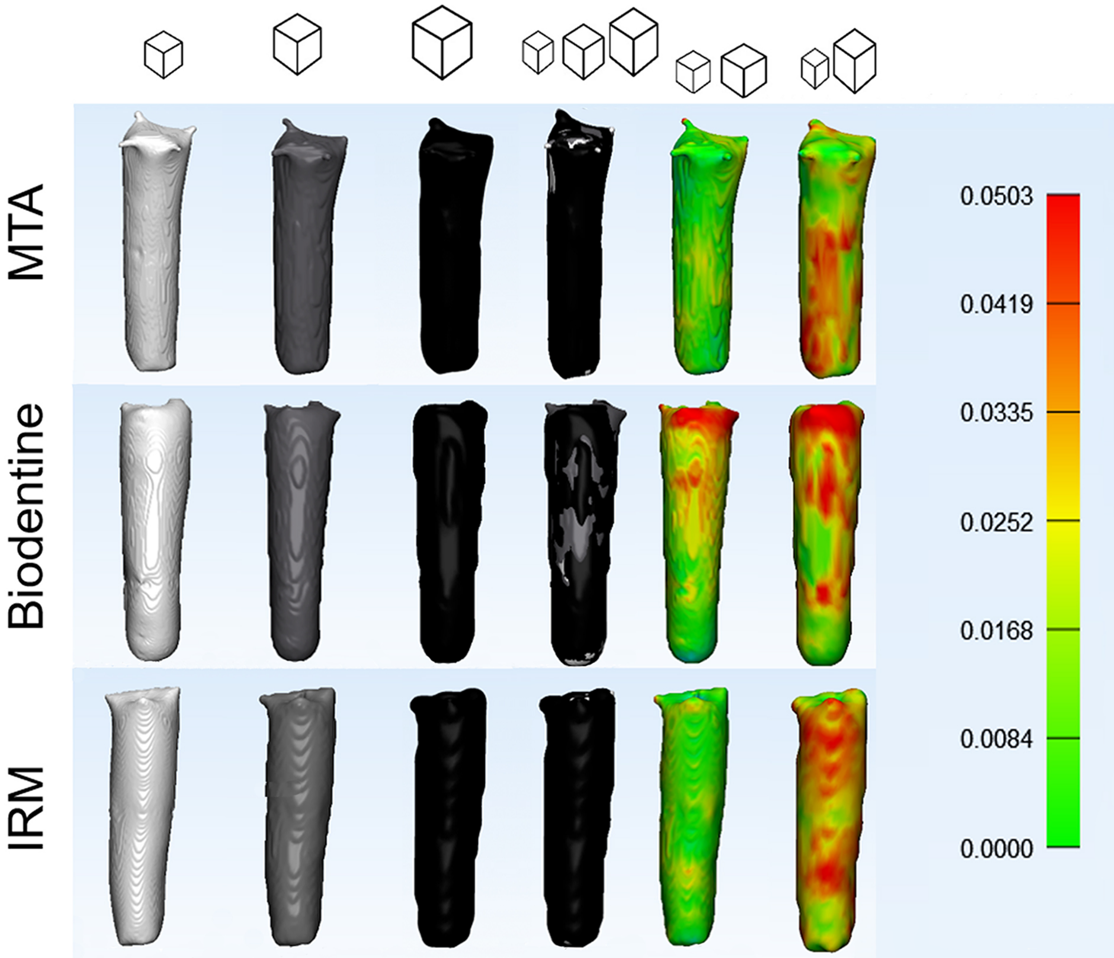

Fig. 2. 3D models of MTA, Biodentine and IRM at 5 μm (small cube representing the smaller voxel size; white), 10 μm (medium cube representing the medium voxel size; gray), 20 μm (large cube representing the larger voxel size; black), and after superimposition between 5 and 10 μm, and 5 and 20 μm. The color map shows the increase in volume (mm) after superimposition.

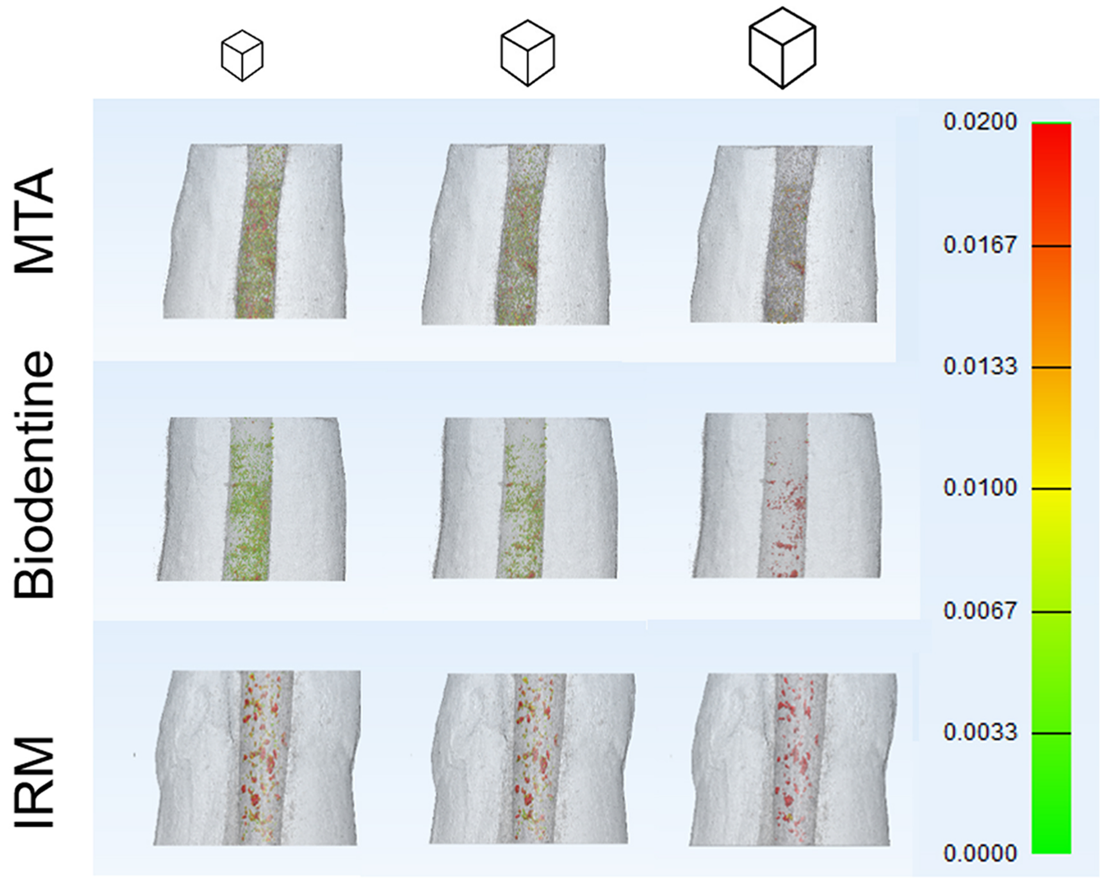

Fig. 3. 3D models representing the porosity of the MTA, Biodentine and IRM cements at 5 μm (small cube representing the smaller voxel size), 10 μm (medium cube representing the medium voxel size) and 20 μm (large cube representing the larger voxel size). The color map shows the thickness (mm) of the pores.

3.8 Publicação 8*

Effect of micro-CT-based voxel size on assessment of root-end filling materials

Abstract

The aim of this study was to assess the impact of micro-CT-based voxel size on analysis of the material/dentine interface voids and wall thickness of different endodontic cements. Following root-end resection and apical preparation, maxillary premolars were filled with MTA, Biodentine and IRM (n = 24). The samples were scanned in micro-CT SkyScan 1272 (Bruker, Kontich, Belgium) and the cement/dentine interface and thickness of the materials were evaluated at voxel sizes of 5, 10 and 20 µm. Data were submitted to ANOVA/Tukey tests and the degree of agreement between the different voxel sizes was evaluated using Bland and Altman method ($\alpha = .05$). All the materials showed an increase in their thickness from 5 to 10 and 20 µm ($p < .05$). When evaluating the interface voids, the materials were similar at 5 µm ($p > .05$), while at 10 and 20 µm Biodentine showed the lowest percentage of voids ($p < .05$). A decrease in the interface voids was observed for MTA and IRM at 20 µm, while Biodentine showed differences among all voxel sizes ($p < .05$). The Bland Altman plots for comparison among voxel sizes showed the largest deviation when comparing images between 5 and 20 µm. In conclusion, there was an impact of voxel sizes on micro-CT evaluation of thickness and interface voids of endodontic materials. All the cements had an increase in their thickness and a decrease in the void's percentage, mainly when evaluating images at 20 µm.

KEYWORDS

dental materials, endodontics, micro-CT, root canal filling, voxel

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1 INTRODUCTION

Mineral trioxide aggregate (MTA) has been widely used in endodontics for several applications, due to its clinically successful long-term results (Koseoglu et al., 2017). However, to overcome its drawbacks, as long setting time, tooth discoloration and handling characteristics, a range of bioactive endodontic cements have been manufactured (Parirokh, Torabinejad, & Dummer, 2018). Among such materials, Biodentine (Septodont, Saint Maur des Fossés, France) was developed as a promising calcium silicate-based material with similar clinical applications to MTA (Ballal, Ulusoy, Chhaparwal, & Ginjupalli, 2018). Biodentine presents adequate biological properties (Akbulut, Uyar Arpaci, & Unverdi Eldeniz, 2016) and proper viscosity (Karadas & Atici, 2020).

The potential of the root-end filling materials to adhere to surrounding dental tissue is desired in order to provide a hermetic obturation, avoiding bacterial leakage and contamination of periapical tissues (Biocanin et al., 2018). Therefore, the evaluation of the dentin/materials interaction is an important issue in endodontics (Kucukkaya Eren, Gorduysus, & Sahin, 2017), since gaps between the dentin and material could jeopardize an adequate sealing (Aksel, Arslan, Purali, Uyanik, & Nagas, 2019). As a consequence, the selection of an appropriate material is a challenge in clinical practice, mainly because there is little information about adaptation of materials to dentin (Karadas & Atici, 2020), which is critical for insuring favourable outcome of treatment.

Micro-computed tomography (micro-CT) is a nondestructive tool, which presents great accuracy for evaluation of root canal filling quality and void presence (Orhan et al., 2018). The materials/dentine interface evaluation provided by 3D information using micro-CT has been the focus of many studies, which presented a significant variation in reported voxel sizes for scanning (Almeida et al., 2017; Biocanin et al., 2018; Gandolfi, Parrilli, Fini, Prati, & Dummer, 2013; Kriznar, Zanini, & Fidler, 2019). The voxel size is one of the factor that can impact on image quality (Bouxsein et al., 2010). Consequently, the quality of the image could affect the segmentation technique, and the quantitative and qualitative measurements (Maret et al., 2014). Scanning with low resolution can cause an underestimation of material density due to partial-volume effects and overestimation of object thickness (Bouxsein et al., 2010). Moreover, high resolution is crucial to obtaining accurate results in the calculation of voids, which can be underestimated in higher voxel sizes

(Orhan et al., 2018). In spite of the smallest voxel size should be chosen for most of the studies, higher resolution scans require longer acquisition times, besides generate large data sets (Bouxsein et al., 2010).

Since there is a lack of consistency on endodontic materials evaluation and the potential bias introduced by micro-CT image postprocessing such as resolution variables is generally unknown (Jirik et al., 2018), our aim was to evaluate by micro-CT the material/dentine interface voids, and thickness of different endodontic cements at voxel sizes of 5, 10 and 20 μm. The null hypothesis was that there is no difference between the different voxel sizes on evaluation of the materials.

2 MATERIALS AND METHODS

2.1 Specimen selection and preparation

After approval by the Institutional Ethics Committee (#CAAE: 9779617.5.0000.5416), extracted two-rooted maxillary premolars were selected. Any teeth with previous root canal treatments, cracks, or perforations were excluded from the experiment. A digital radiography system (Kodak RVG 6100, Kodak Dental Systems, NY, USA) was used to confirm the inclusion criteria of teeth with similar morphology and to perform a homogeneous distribution of the samples. The initial sample selection was performed using a specific software G* Power (3.1.7 for Windows, Heinrich Heine, Universität Dusseldorf, Germany). The t-test for two independent groups was used with an Alpha type error of 0.05 and Beta power of 0.95, based on a previous study (Gandolfi et al., 2013). The teeth were randomly assigned to 3 groups according to the root-end filling materials (n = 24).

Root end resections were performed at 90° to the long axis of the both roots of the selected premolars, using a high-speed hand-piece under constant water spray irrigation. To standardize for uniformity of the length of the root end resection, the teeth were fixed in condensation silicone (Oranwash, Zhermack SpA, Badia Polesine, Italy), and efforts were made to use the burs exactly 3 mm from the apex. After the apicectomy, 3.0 mm deep cavities were prepared using an ultrawave (Ultrawave XS, Ultradent, South Jordan, Utah) appliance at a medium-power setting (50%) with distilled water irrigation. A feather-like, back-and-forth motion were applied with slight coronal pressure using an ultrasonic retrotip T1F-R (CVD-Vale, São José dos

Campos, SP, Brazil). The lengths and radii of the retro-tips (3 mm) determined the depth of the root-end cavities and their final diameter.

MTA (Angelus, Londrina, PR, Brazil), Biodentine (Septodont, Saint Maur des Fosses, France) and IRM (Dentsply DeTrey, Konstanz, Germany) were mixed according to the manufacturers' instructions and the cavities were filled with the materials. The samples were kept in an oven at 37°C and relative humidity for 24 hours. All the procedures were performed with the aid of magnifying glass under 3.5x magnification (Bio-Art, Sao Carlos, SP, Brazil), by a single operator who was previously trained and calibrated.

2.2 Image acquisition and reconstruction

All samples were scanned using SkyScan 1272 micro-CT system (SkyScan, Bruker, Kontich, Belgium). The parameters for image acquisition and correction settings during reconstruction of the images using NRecon software (V1.6.10.4; Bruker, Kontich, Belgium) are described in Table 1. All data sets were exported using the DICOM file format with an isotropic voxel size of 5 μm.

2.3 Image segmentation and analysis

For the propose of voids evaluation, the reconstructed images were segmented differentiating material and dentine using an adaptive thresholding in CTAn software (V1.15.4.0; Bruker, Kontich, Belgium) at 5 μm, and after resizing the images to 10 and 20 μm. The percentage of voids at the interface between the dentin surface of the root canal walls and the filling materials were evaluated based on the method described in a previous study (Gandolfi et al., 2013). The 3D distribution of the interface voids in a pre-defined volume of interest (VOI) was calculated at 5, 10 and 20 μm using CTAn software. 3D models of the voids were created and exported to Materialise 3-matic (Materialise; Leuven, Belgium) to show the voids' thickness.

Data sets were also imported for segmentation at 5, 10 and 20 μm into a dedicated tool developed in MeVisLab (MeVis Research, Bremen, Germany) (EzEldeen, Van Gorp, Van Dessel, Vandermeulen, & Jacobs, 2015) in order to quantify the wall thickness of the materials. After segmentation by MeVisLab, morphological analysis was performed in Materialise 3-matic and the thickness of the materials was measured.

2.4 Statistical analysis

All data were analyzed with statistical software package GraphPad Prism 7.00 (GraphPad Software, La Jolla California USA). The normality of the data was tested using the D'Agostino & Pearson test. One-way ANOVA/Tukey were used to test for statistical differences between the different materials and voxel sizes. The degree of agreement between the different voxel sizes was evaluated using Bland and Altman method (MedCalc Software, Ostend, Belgium). Bland and Altman analysis is based on quantifying the agreement between two quantitative measurements, achieved by analyzing the bias between the mean differences, and estimating an agreement interval (Giavarina, 2015). The level of significance was set at 5%.

3 RESULTS

A box plot graph representing the material's thickness is presented in Figure 1. All the materials showed an increase in their thickness from 5 to 10 and 20 μm ($p < .05$). The evaluation of materials using 3-matic software can be visualized in Figure 2.

When evaluating the material/dentin interface by CTAn software, the cements showed a decrease in the percentage of voids when using greater voxel size (Table 2 and Figure 4). The percentage of interface voids was similar between the materials at 5 μm, while at 10 and 20 μm Biodentine showed lower values than MTA and IRM ($p < .05$), which were similar ($p > .05$). There was no difference between 5 and 10 μm for MTA and IRM ($p > .05$) and the voxel size of 20 μm was statistically different ($p < .05$). Biodentine showed significant differences between all resolutions ($p < .05$). The Bland Altman plots for comparison among voxel sizes showed the largest deviation when comparing images with 5 and 20 μm voxel sizes (Figures 3 and 5).

4 DISCUSSION

Although micro-CT has been extensively used for characterization of dental materials, specific guidelines for acquisition, processing, and analysis of the images have not been defined yet (De Souza et al., 2013). Since the voxel size can have a significant impact on quantitative analysis using micro-CT, studies of image parameter dependency are essentials to provide a background information to support the choice of scan protocols and allow inter-study comparisons (Cooper, Turinsky, Sensen, & Hallgrimsson, 2007). Therefore, the current study was undertaken to determine the voxel size dependency to accurate evaluation of material/dentine interface and thickness of endodontic materials using 3D micro-CT imaging. The Bland and Altman plot was used to compare the voxel sizes in regard to correlation and differences, by analysis of the same variable. The Bland and Altman analysis defines only the intervals of agreement, without determining whether or not the limits are acceptable (Giavarina, 2015). However, the differences observed on investigations as the current study serve to highlight the effects of decreasing resolution on the accuracy of the micro-CT quantification (Jirik et al., 2018).

The wall thickness of the materials was evaluated using 3-matic program (Materialise, Leuven, Belgium). According to the manufacturer, this analysis refers to the distance between one surface of the material and its opposite sheer surface. The wall thickness analysis takes a voxel-based approach. It is comparable to pixels in 2D, but used in 3D space, and represented by a cube volume. The analysis tool splits up the 3D model in a series of voxel or cube-shaped volumes. Based on the 3D triangulated surfaces, a finite element mesh is generated, which is commonly used in the medical field, aiming to develop and validate a workflow to simulate a treatment prior to the intervention (Bosmans, Famaey, Verhoelst, Bosmans, & Vander Sloten, 2016). Our results showed that the thickness of the materials increased when we increased the voxel size. It probably occurred because a lower spatial resolution induces errors in the calculation of the 3D images, creating an overestimation of some parameters, increasing the percent volume (Kim et al., 2015). When the resolution of the image decreases a partial volume effect is expected, which is an inevitable phenomenon that occurs when neighboring voxels includes multiple materials. The partial volume does not have the same attenuation value as any of the individual materials inside the voxel, and voxels at the external surface of the sample can contain both volumes occupied by the two materials. Therefore, completely accurate object volume measurement is not possible (Choi, Choi, & Yeo, 2018; Marinozzi et al., 2013). The partial volume effect depends on the size of the target object, the contrast between the object and background, the spatial resolution, reconstruction methods and smoothing filters used (Ferretti et al., 2012).

Differently than what occurred in the analysis of thickness, the assessment of the interface voids showed in general a decrease of values when we increased the voxel size. Our findings showed no difference between the materials regarding their interface voids when evaluating images at 5 μm, in agreement with previous studies (Kucukkaya Eren et al., 2019; Kucukkaya Eren et al., 2017; Ozturk et al., 2019).

However, at 10 and 20 μm Biodentine showed lower voids values than MTA and IRM. These values occurred since the micro-CT images is limited by the dimensions of the target object. When the resolution exceeds the dimensions of micropores or very thin structures, they are not correctly visualized (Kerckhofs, Schrooten, Van Cleynenbreugel, Lomov, & Wevers, 2008).

When reducing spatial resolution from 5 to 10 and 20 μm, our results showed a reduction in percentage of interface voids of 6 and 29% for MTA, 42 and 78% for Biodentine, 2 and 35% for IRM. The highest values for Biodentine can be justified by the smaller voids present in its interface, as observed in Figure 4. This finding could be justified since MTA has difficult handling characteristics as a direct result of its granular consistency (Ber, Hatton, & Stewart, 2007), while Biodentine has a hydro-soluble polymer incorporated to the water, which improve its handling properties (Camilleri, Sorrentino, & Damidot, 2013). Moreover, Biodentine present small and optimized particle size distribution in its powder (Camilleri et al., 2013). Therefore, these characteristics could explain the low diameter of pores or voids in the cement-dentin interface for Biodentine (Biocanin et al., 2018). A previous study (Orhan et al., 2018) compared different voxel sizes using nano-CT (1.5 and 5.0 μm) and micro-CT (5.2, 8.1, 11.2, and 16.73 μm) for the evaluation of root canal filling voids. The authors suggested a voxel resolution of 11.2 μm as cut-off value in micro-CT and nano-CT imaging, corroborating another study (De Souza et al., 2013) stating that 9.52 μm could be considered the optimal micro-CT-based resolution for quantification of porosity in different materials. These findings are in agreement with our results for MTA and IRM.

Within the limitation of the current study, our results showed that smaller voxel sizes are preferable for evaluation of materials thickness and interface voids of cements, rejecting our null hypothesis. Although a lower resolution could allow these assessments it is important to consider that measurements are only approximations of the real values (Cooper et al., 2007).

5 CONCLUSION

There was an impact of voxel sizes on micro-CT evaluation of thickness and interface voids of endodontic materials. All the cements had an increase in their thickness and a decrease in the void’s percentage when the voxel size was bigger (20 μm).

CONFLICT OF INTEREST

The authors deny any conflict of interest related to this study.

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Tables

Table 1 - Parameters for micro-CT acquisition and reconstruction of the samples

Voxel Size	5 µm
Source voltage	100 kV
Source current	100 µA
Rotation	180º
Frame averaging	4
Rotation step	0.2
Ring Artifact Correction	5
Beam Hardening Correction	25
Smoothing	4

Table 2 - Interface voids values (%) using different voxel sizes (mean and standard deviation) observed in endodontic materials.

Interface Gaps/Voids (%)	MTA	Biodentine	IRM
5 µm	2,07 (1,23)^{Aa}	2,63 (1,62)^{Aa}	2,49 (1,32)^{Aa}
10 µm	1,94 (1,15)^{Aa}	1,11 (1,12)^{Bb}	2,43 (1,82)^{Aa}
20 µm	1,47 (1,07)^{Ba}	0,56 (0,46)^{Cb}	1,60 (1,53)^{Ba}

^{ABC}Different capital letters in the same column indicate statistically significant difference between the voxel sizes ($p < 0.05$).
^{abc}Different lower case letters in the same line indicate statistically significant difference between the materials ($p < 0.05$).

Figures

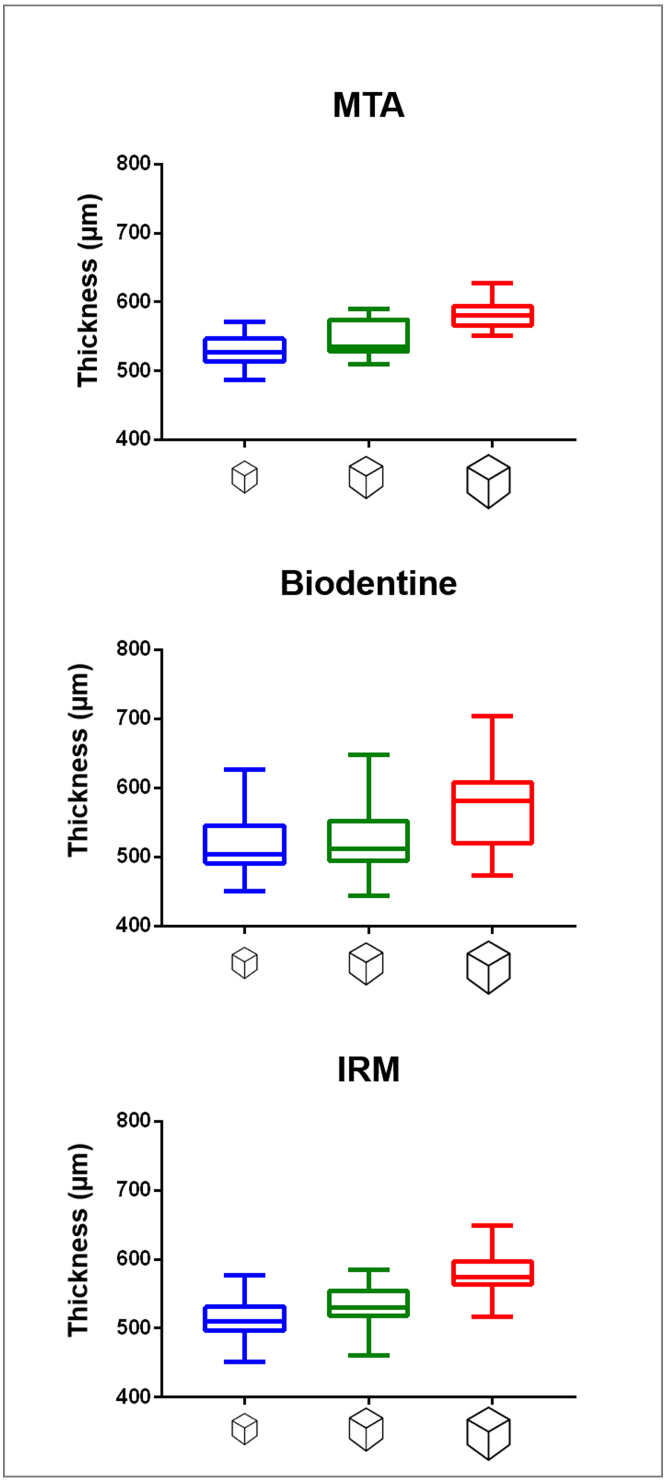

Figure 1 - Box plot graph representing the material's thickness (µm) at 5 (small cube representing the smaller voxel size), 10 (medium cube representing the medium voxel size) and 20 µm (big cube representing the bigger voxel size) for MTA, Biodentine and IRM. Statistical differences were observed in their thickness from 5 to 10 and 20 µm.

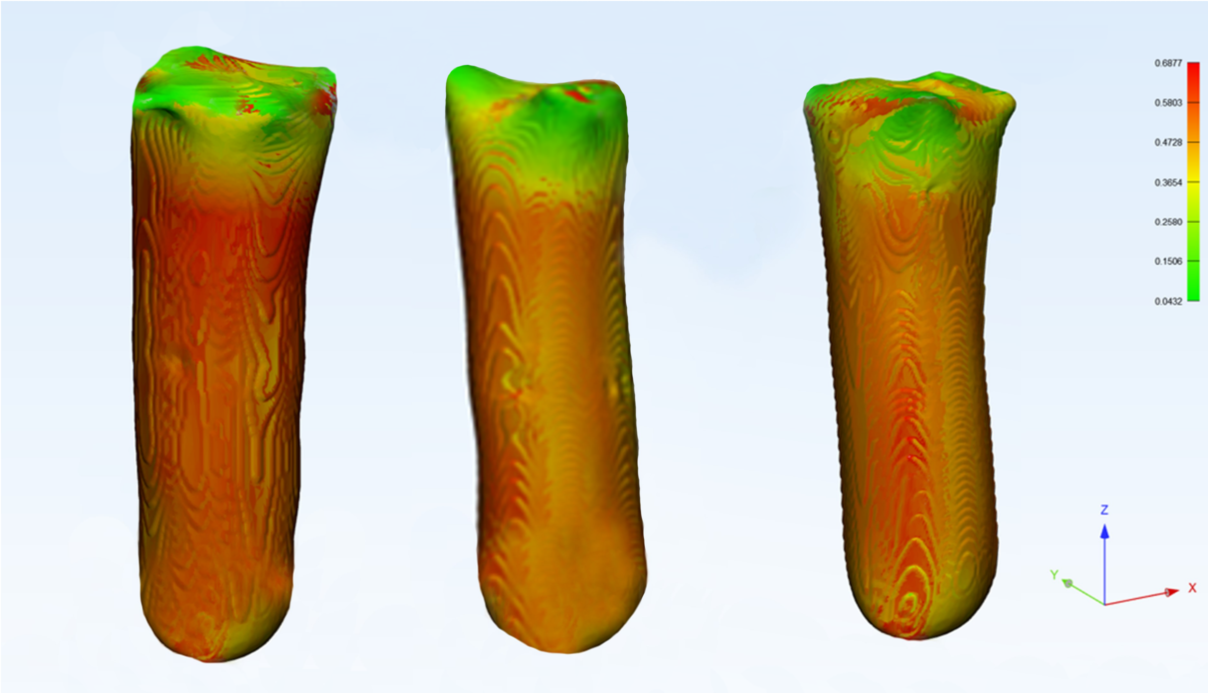

Figure 2 - 3D models representing the wall thickness analysis by 3-matic software of MTA, Biodentine and IRM at 5 µm. The color map shows the thickness (mm) of the materials.

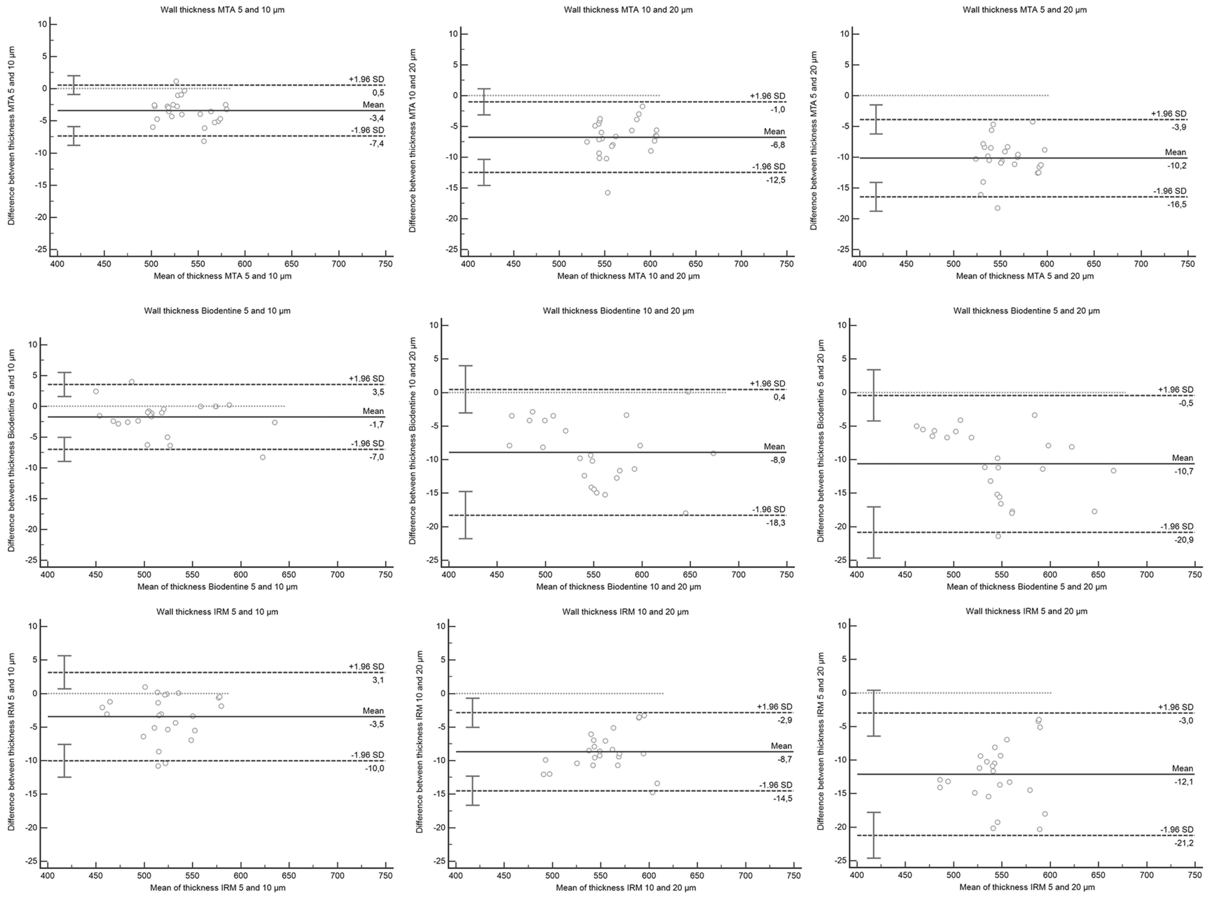

Figure 3 - Bland-Altman plots for voxel sizes comparison of agreement regarding wall thickness analysis. The difference between the measurements is plotted against their mean. The graphs represent MTA, Biodentine, and IRM comparing 5 and 10 µm, 10 and 20 µm, 5 and 20 µm, respectively. SD, standard deviation.

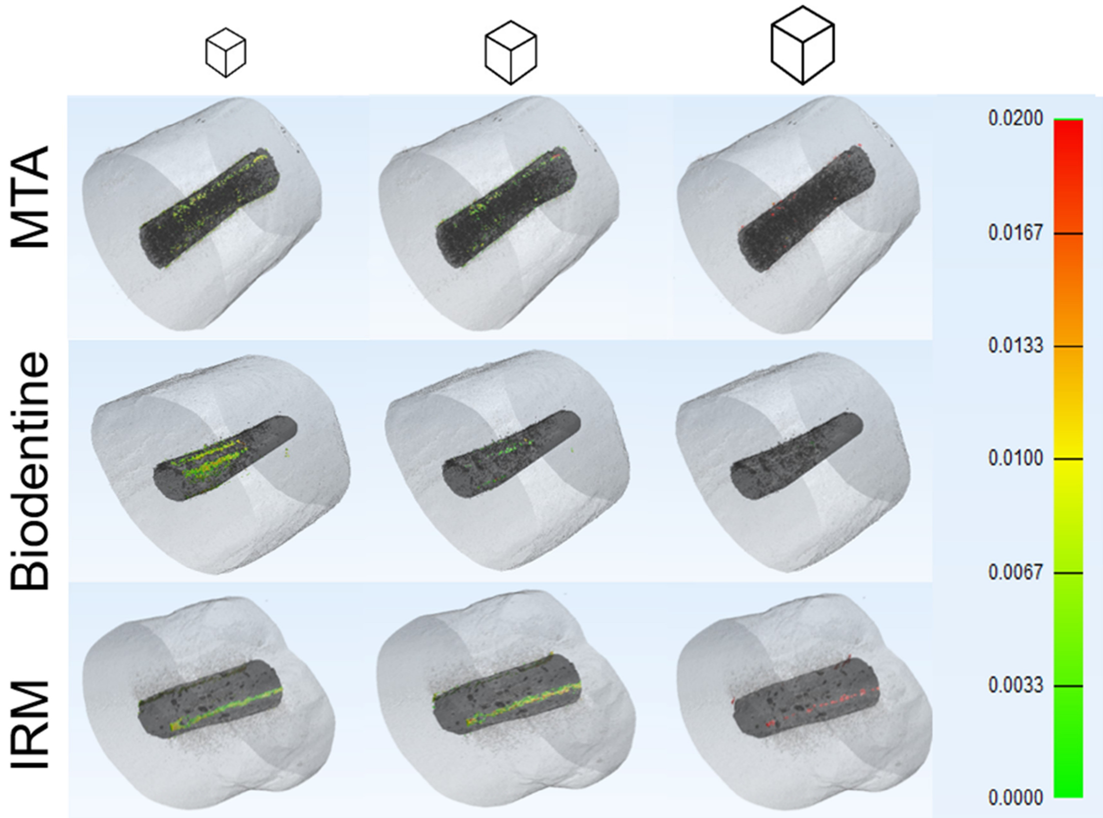

Figure 4 - 3D models representing the interface gaps/voids of the cements MTA, Biodentine and IRM at 5 (small cube representing the smaller voxel size), 10 (medium cube representing the medium voxel size) and 20 μm (big cube representing the bigger voxel size). The color map shows the thickness (mm) of the voids.

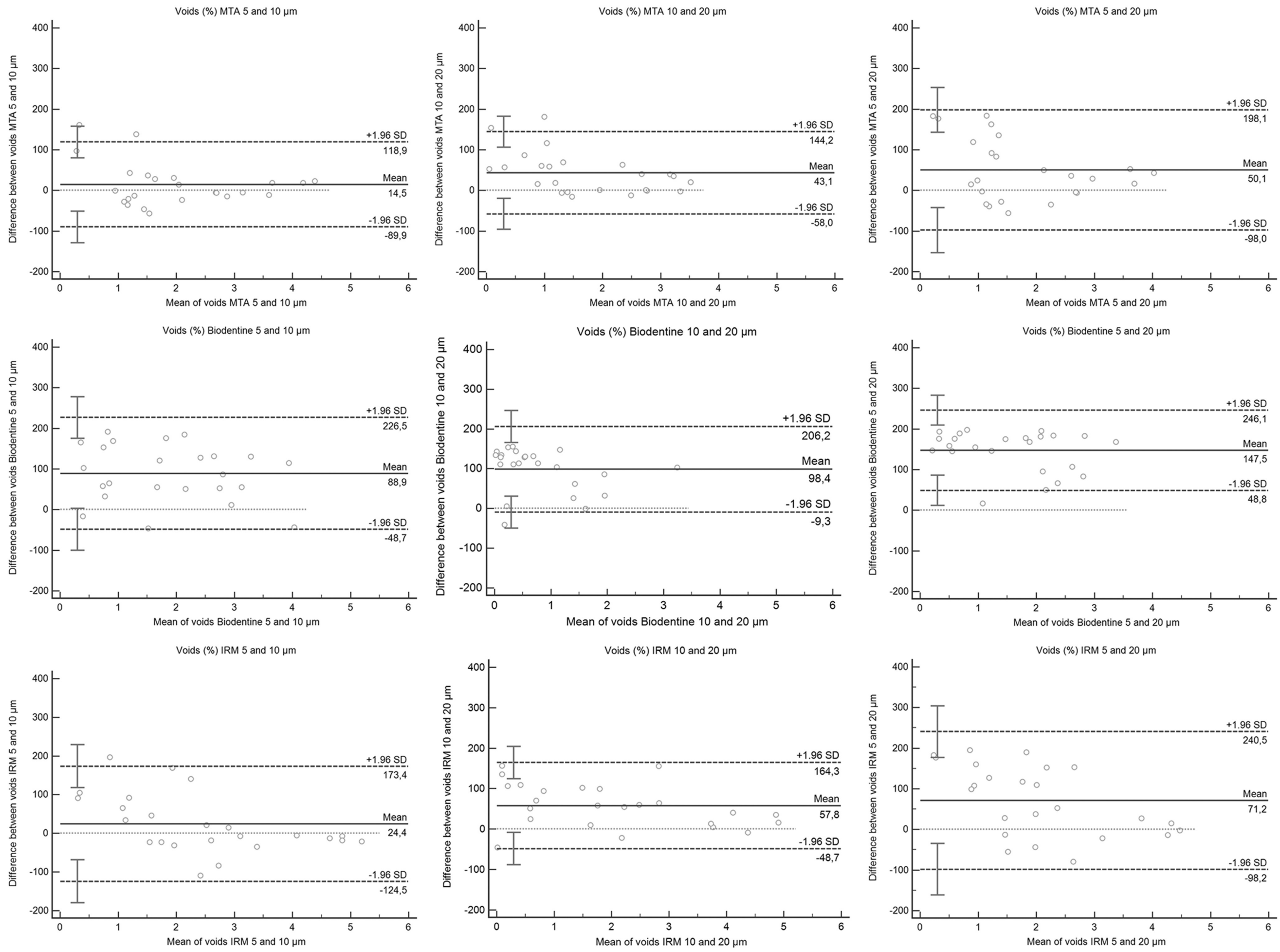

Figure 5 - Bland-Altman plots for voxel sizes comparison of agreement regarding interface gaps/voids of the cements. The difference between the measurements is plotted against their mean. The graphs represent MTA, Biodentine, and IRM comparing 5 and 10 µm, 10 and 20 µm, 5 and 20 µm, respectively. SD, standard deviation.

4 DISCUSSÃO

As propriedades físico-químicas de solubilidade e alteração dimensional são avaliadas pelas normas ISO e ADA. O teste de estabilidade dimensional é realizado por meio de mensuração linear em um único plano, com acurácia de avaliação (±1 μm), podendo ser insuficiente para registrar pequenas alterações[16]. A solubilidade é avaliada pela diferença de massa, em gramas, antes e após a imersão em água[7], o que também pode ser insuficiente para análise do comportamento volumétrico do material. Além disso, a solubilidade é avaliada pelas normas ISO após um período de 24 horas. No entanto, períodos maiores de análise são utilizados, a fim de avaliar o comportamento dos materiais ao longo do tempo[8-12,14]. O período de 7 dias tem sido amplamente utilizado[89-92].

A utilização da microtomografia computadorizada (micro-CT) no presente estudo possibilitou análise volumétrica (em mm³) dos materiais, aperfeiçoando o teste convencional de análise da alteração dimensional. Ainda, a análise após diferentes períodos de imersão em água destilada complementa a avaliação de solubilidade dos materiais, e possibilita maior entendimento do comportamento dos materiais como um todo[6,13,45,75] em períodos maiores que 24 horas.

Resultados dos subprojetos 1 e 2 mostraram que AH Plus apresenta aumento de volume após 7 dias de imersão em água destilada. Fill Canal apresentou aumento de volume quando avaliado nos moldes de resina, entretanto, quando o material foi colocado em contato direto com a água destilada no subprojeto 2, mostrou perda volumétrica. O aumento volumétrico observado para AH Plus e Fill Canal (que é um cimento à base de óxido de zinco e eugenol, semelhante ao EndoFill) podem ter ocorrido em função da expansão que ocorre nesses materiais quando avaliados pelo teste de alteração dimensional[4,18,20,21,27,93,94]. Esses cimentos também apresentam solubilidade dentro dos padrões estabelecidos pela ISO, com valores inferiores a 3%[8,17,18,21,25-33,38,39,95], como observado na perda volumétrica, quando ocorreu após 30 dias para AH Plus e no subprojeto 2 para Fill Canal. Para Sealapex, as reduções volumétricas observadas em todas as amostras avaliadas podem ser justificadas em função da alta solubilidade desse cimento[8,27,38,39], além de sua contração quando avaliado pelo teste de estabilidade dimensional[27].

Para os cimentos reparadores, nossos resultados dos subprojetos 1 e 2 mostraram que Biodentine perdeu volume, independentemente dos espécimes avaliados. Além disso, este material mostrou redução de sua espessura quando avaliado no interior da cavidade retrógrada no subprojeto 5. IRM ganhou volume após 7 dias de imersão no subprojeto 1, enquanto quando avaliado no subprojeto 2 apenas o material, sem a cavidade de resina, bem como após 30 dias no subprojeto 1 houve uma perda volumétrica, mostrando que houve uma diminuição de volume conforme o aumento do contato com o meio aquoso, semelhante ao que ocorreu para Fill Canal, que também é um cimento à base de óxido de zinco e eugenol.

Nossos resultados mostram baixa variação volumétrica para o MTA nos subprojetos 1, 2 e 5, o que pode ser justificado em função da baixa solubilidade desse material[13,59]. Ainda, MTA apresenta expansão no teste de estabilidade dimensional[13,96], em função de sua reação de hidratação[97]. Esses resultados também justificam a adaptação marginal desse material observada no subprojeto 5. A perda volumétrica e morfológica do Biodentine pode ser relacionada à sua alta solubilidade[11,68,89,98] e presença do policarboxilato em sua composição[89]. A solubilidade pode contribuir para o aumento da porosidade e dos vazios na interface material/dentina, que foram avaliados no subprojeto 5. Para IRM, a diminuição progressiva do ganho volumétrico inicial acontece devido à sua solubilidade que pode ocorrer pela perda contínua de eugenol, causando um efeito de lixiviação[99]. Entretanto, quando avaliado no interior da cavidade retrógrada no subprojeto 5 este material mostrou ser estável.

Outra observação importante do nosso estudo foi que os tamanhos dos corpos de prova não apresentaram uma influência significativa nas alterações volumétricas dos materiais. Entretanto, o tempo de imersão em água destilada mostrou uma tendência em aumentar a perda de volume dos mesmos.

Cimentos à base de silicato de cálcio são desenvolvidos para emprego como biomateriais endodônticos, em função de sua biocompatibilidade, bioatividade, não-citotoxicidade e não-genotoxicidade[100]. TotalFill BC Sealer apresenta propriedades físico-químicas aceitáveis, mas demonstra solubilidade[45,101,102], como foi possível observar nos nossos resultados do subprojeto 4. Sealer Plus BC e Bio-C Sealer foram desenvolvidos e recentemente introduzidos no mercado. Estudos têm mostrado propriedades físico-químicas satisfatórias para Sealer Plus BC[49] e Bio-C Sealer[43], embora apresentem solubilidade acima do recomendado.

Embora a alta solubilidade tenha se mostrado como uma desvantagem de cimentos à base de silicato de cálcio, a imersão desses cimentos em fluidos corporais simulados fornece maior similaridade com a aplicação clínica e tem mostrado menor solubilidade para materiais bioativos[103]. Desta forma, nosso estudo propôs avaliar a solubilidade e alteração volumétrica desses materiais também em PBS. Nossos resultados mostraram que tais materiais apresentaram uma maior solubilidade e perda de volume quando imersos em água destilada, e menor em PBS. Além disso, apesar dos materiais à base de silicato de cálcio apresentarem altos valores de solubilidade, todos mostraram baixa alteração volumétrica, mesmo quando imersos em água destilada. Tais observações podem ser justificadas pela absorção de fluidos desses cimentos, que pode levar a uma estabilidade volumétrica[75].

Outra importante propriedade físico-química que deve ser avaliada nos cimentos endodônticos é o escoamento, visto que a fluidez desses materiais é importante para a penetração e possível preenchimento das irregularidades dos canais radiculares e cavidades retrógradas[3,4].

Nossos resultados do subprojeto 3, avaliando o escoamento convencional dos cimentos obturadores mostrou altos valores para AH Plus e Sealapex, corroborando Almeida et al.[5], Tanomaru-Filho et al.[104] e Viapiana et al.[27] Não houve diferença significante entre os materiais quando avaliados em milímetros e em área (mm²). Chang et al.[105] não observaram diferença no escoamento dos cimentos AH Plus e Sealapex utilizando a mesma metodologia do presente estudo. Entretanto, ao avaliar a viscosidade desses cimentos utilizado um reômetro de deformação controlado, os valores foram maiores para Sealapex, sugerindo limitações no teste convencional. Almeida et al.[5] avaliaram o escoamento de cimentos obturadores utilizando a técnica convencional e compararam com a habilidade de preencher canais laterais. Embora os autores tenham observado valores abaixo do recomendado pela ISO para Endométhasone e Sealapex, ambos mostraram alto percentual de preenchimento, sugerindo ausência de correlação entre escoamento e preenchimento.

Para os cimentos reparadores, IRM foi o material que apresentou o maior escoamento em milímetros, concordando com estudo prévio[80]. Entretanto, não houve diferença entre os cimentos quando comparado o escoamento em área (mm²), de forma semelhante com o que ocorreu para os cimentos obturadores. Tanomaru-Filho et al.[80] avaliaram o escoamento convencional dos cimentos MTA, Biodentine e óxido de zinco e eugenol, em comparação com o escoamento e capacidade de preenchimento desses cimentos quando avaliados em micro-CT.

Embora o cimento de óxido de zinco e eugenol tenha apresentado melhor escoamento, Biodentine mostrou melhor capacidade de preenchimento, mostrando mais uma vez que escoamento e preenchimento não necessariamente se correlacionam, conforme citado em estudos acima sobre os cimentos obturadores.

A avaliação do escoamento e preenchimento dos materiais retrobturadores utilizando diferentes modelos de teste em micro-CT mostraram que materiais com maior escoamento apresentam menor capacidade de preenchimento, sendo que uma maior altura das canaletas também influenciou negativamente o preenchimento dos espaços pelos cimentos. Isso mostra a necessidade de uma padronização nas técnicas de avaliações em micro-CT, para permitir a comparação de resultados observados em diferentes estudos.

Ainda sobre padronização, nosso subprojeto 5 reforça esta necessidade quanto aos parâmetros de escaneamento e formas de análise de outras propriedades físico-químicas de materiais endodônticos. Foi possível observar que houve um aumento no volume e espessura dos materiais conforme aumentamos o voxel de 5 para 20 µm. Em contrapartida, a porosidade no interior dos materiais, bem como o vazio na interface diminuíram progressivamente. Além disso, nosso estudo mostrou que variações obtidas nos resultados encontrados em diferentes softwares foram mais comuns quando utilizando um maior tamanho de voxel. Isso nos leva a crer que a comparação entre diferentes estudos utilizando micro-CT deve ser minuciosamente realizada, levando em consideração a resolução em que as imagens foram obtidas, bem como os softwares para interpretação dessas imagens.

5 CONCLUSÃO

Com os resultados do presente estudo, podemos concluir que:

Subprojeto 1- O tempo de imersão em água destilada tende a aumentar a perda de volume de cimentos endodônticos. Portanto, períodos maiores de imersão possibilitam avaliação confiável do comportamento dos materiais ao longo do tempo.

Subprojeto 2- O tamanho das amostras não afetou a porcentagem de alteração volumétrica dos cimentos, podendo o pesquisador utilizar menores quantidades de materiais sem afetar a acurácia dos resultados.

Subprojeto 3- Canaletas com maior altura e materiais com maior escoamento proporcionaram menor capacidade de preenchimento para cimentos retrobturadores. As variações nos modelos de teste não influenciaram a avaliação do escoamento e preenchimento dos cimentos obturadores.

Subprojeto 4- Embora cimentos obturadores à base de silicato de cálcio apresentem maior solubilidade que AH Plus, tais materiais mostram baixas alterações volumétricas. Armazenamento em PBS reduz a solubilidade e perda volumétrica de cimentos à base de silicato de cálcio.

Subprojeto 5- MTA apresenta estabilidade dimensional e volumétrica, apesar de maior porosidade que Biodentine após imersão em PBS. Maiores resoluções das imagens e radiopacidade dos materiais aumentam a confiabilidade dos resultados voltados para propriedades físico-químicas de cimentos reparadores. Desta forma, com base nos resultados do presente estudo podemos sugerir a utilização do tamanho de voxel de 5 µm quando associando os programas MeVisLab e Materialise 3matic para avaliação do volume e espessura de cimentos retrobturadores. Quando utilizando o programa CTAn para análise do volume dos materiais e da presença de vazios na interface material/dentina, 5 µm deve ser empregado para materiais com menores radiopacidades, como Biodentine e 10 µm para materiais mais radiopacos, como MTA e IRM. Ainda para este software, a porosidade no interior dos materiais é melhor avaliada a 5 µm.

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APÊNDICE A – METODOLOGIA DETALHADA

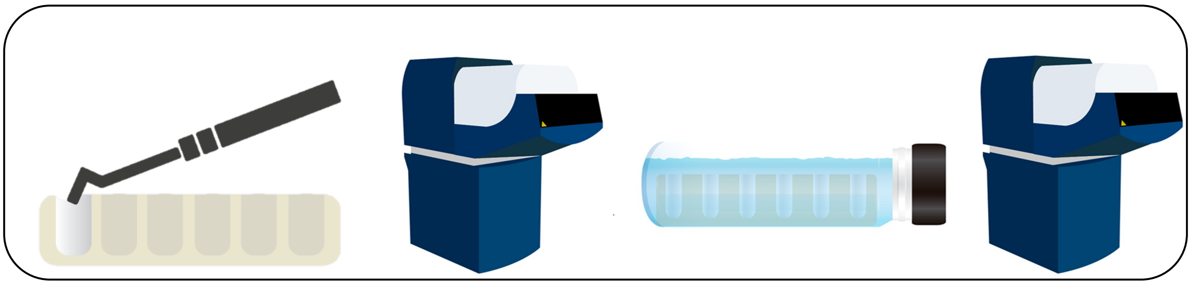
Subprojeto 1: Teste de alteração volumétrica em micro-CT utilizando moldes de resina

O teste de alteração volumétrica foi baseado em em estudo prévio[1]. Para a realização deste teste, modelos de resina acrílica foram confeccionados a partir de moldes metálicos com cavidades de 3 mm de profundidade. As cavidades nos modelos de resina acrílica foram preenchidas com cada um dos materiais logo após sua manipulação, e mantidas por três vezes o tempo de presa de cada material em estufa a 37ºC e em umidade relativa. Os escaneamentos foram realizados logo após a presa dos materiais e nos períodos de 7 e 30 dias utilizando o microtomógrafo SkyScan 1176 (Bruker-microCT, Kontich, Bélgica), sendo mantidos imersos em água destilada. Um esquema ilustrando o processo de avaliação da alteração volumétrica pode ser visto na Figura 1. O volume de preenchimento dos materiais foi calculado em cada período. Os parâmetros de escaneamento foram: voltagem de 50 kv, corrente de 500 µA, 18 µm de tamanho de pixels e rotação de 360°. A reconstrução das imagens foi realizada no programa *NRecon* (V1.6.4,7; Bruker-MicroCT, Kontich, Belgium). Os materiais foram reconstruídos seguindo os seguintes parâmetros:

- AH Plus: 2 para *smoothing*, 60 para *beam hardening,* 2 para *ring*.
- Fill Canal: 2 para *smoothing*, 50 para *beam hardening,* 2 para *ring.*
- Sealapex: 2 para *smoothing*, 50 para *beam hardening* e 2 para *ring.*
- Biodentine: 1 para *smoothing*, 40 para *beam hardening* e 1 para *ring.*
- IRM: 1 para *smoothing*, 50 para *beam hardening* e 1 para *ring.*
- MTA: 1 para *smoothing*, 50 para *beam hardening* e 1 para *ring.*

Os mesmos parâmetros foram usados para o mesmo material nos diferentes períodos. As imagens reconstruídas em cada período foram sobrepostas e salvas nos planos coronal, sagital e transaxial utilizando o programa Data Viewer (V1.5.2.4; Bruker-MicroCT, Kontich, Belgium). A análise das imagens reconstruídas em 3D foi realizada utilizando o programa *CTAn* (V1.11.8; Bruker-MicroCT, Kontich, Belgium).

Figura 1- Ilustração esquematizando o processo de alteração volumétrica do subprojeto 1



As cavidades são preenchidas, escaneadas após a presa, imersas em água destilada e escaneadas novamente após os períodos de 7 e 30 dias.

Fonte: Adaptada de Torres et al.[1,p.377] e Tanomaru-Filho et al.[2,p.2099].

Subprojeto 2: Teste de alteração volumétrica em micro-CT utilizando modelo de teste de solubilidade

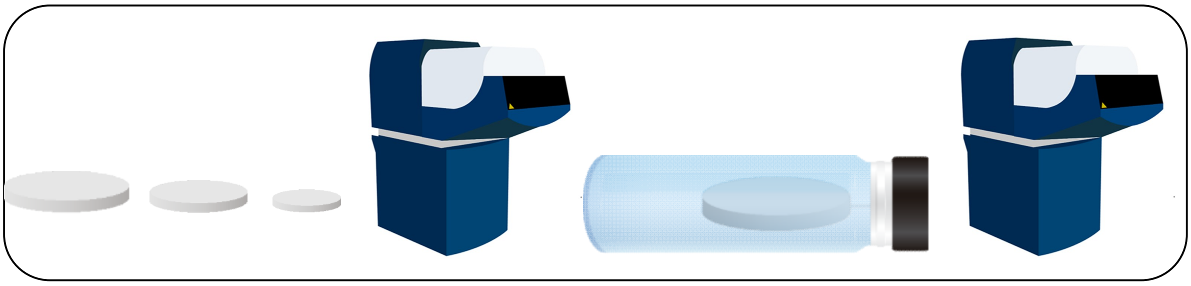
Foram utilizando corpos de prova com 6,30 mm de diâmetro por 1,5 mm de altura; 7,75 mm de diâmetro por 1,5 mm de altura; 9,00 mm de diâmetro por 1,5 mm de altura, baseados em estudo prévio[3]. Os escaneamentos foram realizados logo após a presa dos materiais e após 7 dias de imersão em água destilada, utilizando o microtomógrafo SkyScan 1176 (Bruker-microCT, Kontich, Bélgica), de maneira similar ao descrito no subprojeto 1. Os parâmetros de escaneamento foram: filtro de cobre e alumínio, voltagem de 80 kv, corrente de 300 µA, 17.48 µm de tamanho de voxel e rotação de 360°. A reconstrução das imagens foi realizada no programa *NRecon* (V1.6.4,7; Bruker-MicroCT, Kontich, Belgium), utilizando os seguintes parâmetros:

- AH Plus: 2 para *smoothing*, 40 para *beam hardening,* 2 para *ring*.
- Fill Canal: 2 para *smoothing*, 30 para *beam hardening,* 2 para *ring.*
- Sealapex: 2 para *smoothing*, 30 para *beam hardening* e 2 para *ring.*
- Biodentine: 0 para *smoothing*, 40 para *beam hardening* e 2 para *ring.*
- IRM: 0 para *smoothing*, 50 para *beam hardening* e 2 para *ring.*
- MTA: 0 para *smoothing*, 50 para *beam hardening* e 2 para *ring.*

Os mesmos parâmetros foram usados para o mesmo material nos diferentes períodos. As imagens reconstruídas em cada período foram sobrepostas e salvas nos planos coronal, sagital e transaxial utilizando o programa Data Viewer (V1.5.2.4; Bruker-MicroCT, Kontich, Belgium). A análise das imagens reconstruídas em 3D foi realizada utilizando o programa *CTAn* (V1.11.8; Bruker-MicroCT, Kontich, Belgium).

Um esquema ilustrando o processo de avaliação da alteração volumétrica pode ser visto na Figura 2.

Figura 2- Ilustração esquematizando o processo de alteração volumétrica do subprojeto 2



Os espécimes foram confeccionados em diferentes dimensões, escaneados após a presa e após imersão em água destilada por 7 dias.

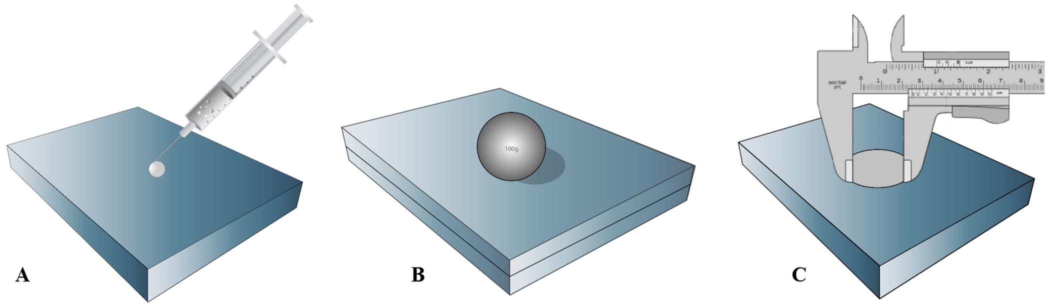
Fonte: Elaboração própria.

Subprojeto 3

Testes de escoamento convencional

O escoamento foi avaliado de acordo com a norma ISO 6876/2012[4]. Após a manipulação do cimento, 0,05 mL do material foi colocado no centro de uma placa de vidro por meio de seringa graduada (n=10). Após 180 ± 5 segundos do início da manipulação, outra placa de vidro (20 g) foi posicionada sobre a placa com o cimento e foi adicionado um peso de 100 gramas sobre a placa superior, mantido por 10 minutos. Após este período, os diâmetros máximo e mínimo do material sobre a placa foram medidos. Quando houve diferença entre os diâmetros inferior a 1 mm, a média foi usada para o teste. Para uma segunda avaliação o material sobre a placa foi fotografado ao lado de uma régua milimetrada. As imagens obtidas foram avaliadas utilizando a ferramenta Image Tool versão 3.0, para obtenção da área de escoamento do material expressa em mm², de acordo com estudo prévio[5].

Figura 3- Ilustração esquematizando teste de escomento segundo normas ISO 6876/2012



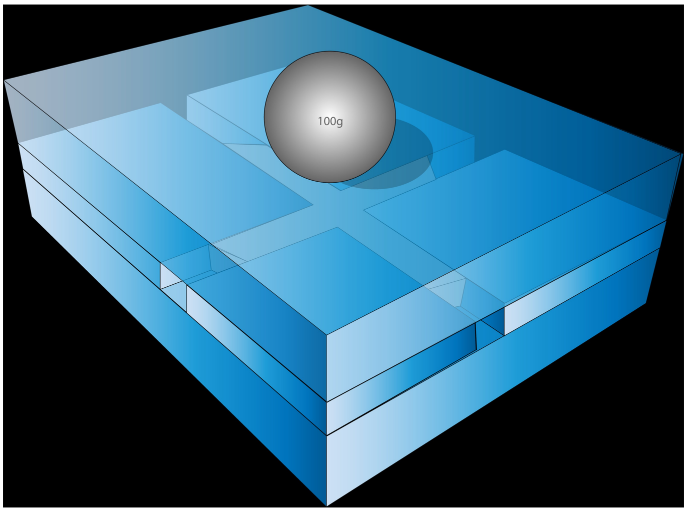
A: 0,05 mL do material é colocado no centro de uma placa de vidro. (B): nova placa de vidro e peso de metal são colocados sobre a placa inferior, totalizando 120 gramas. (C) Após 20 minutos, o peso de metal é removido e o escoamento dos materiais é mensurado por paquímetro digital

Fonte: Torres et al.[6,p.3]

Análise do escoamento por meio de micro-CT

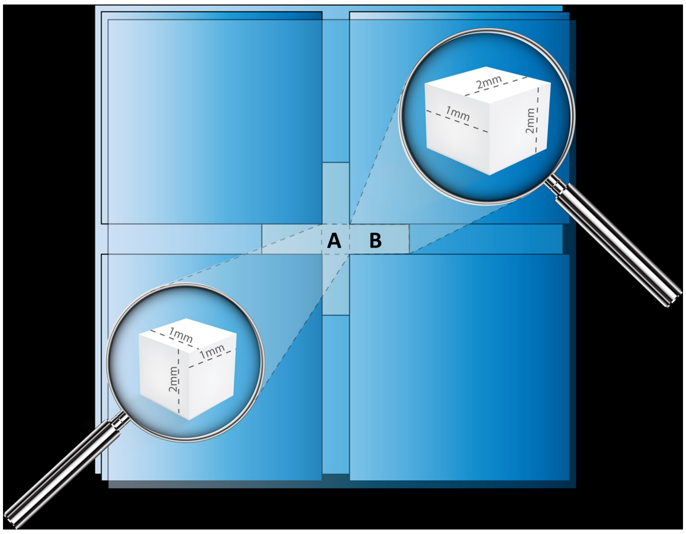
Este teste foi realizado baseado em estudo prévio[7]. Foram confeccionadas placas de vidro com uma cavidade central em diferentes diâmetros para comparação (1 mm de comprimento por 2 mm de altura, 1 mm de comprimento por 1 mm de altura para todos os materiais, além do acréscimo do diâmetro de 2 mm de comprimento por 1 mm de altura para os cimentos reparadores). A partir desta cavidade 4 canaletas foram confeccionadas nos sentidos horizontal e vertical com as mesmas medidas e estendendo-se para os 4 lados. Foram colocados 0,05± 0,005 mL de cada material sobre a cavidade central e sobre eles nova placa de vidro (20 g) e metal (100 g) com massa total de 120 g (Figura 4). A avaliação foi realizada em micro-CT com relação à mensuração do escoamento linear (mm) do material em cada lado da canaleta (horizontal e vertical). A média das 4 medidas foi considerada como escoamento linear para cada avaliação. O preenchimento em volume (mm³) dos materiais na área central foi determinado como Preenchimento Volumétrico Central (PVC). O preenchimento em volume (mm³) dos materiais nas áreas laterais até 2 mm para cada lado a partir da cavidade central foi determinado. A médias dos 4 valores foi considerado Preenchimento Volumétrico Lateral (PVL) para cada análise (Figura 5AB). Os parâmetros de escaneamento foram: voltagem de 90 kv, corrente de 300 µA, 9 µm de tamanho de pixels e rotação de 360°. A reconstrução das imagens foi realizada no programa *NRecon* (V1.6.4,7; SkyScan, Bélgica) e a análise das imagens reconstruídas em 3D foi realizada utilizando o programa *CTAn* (V1.11.8; SkyScan, Bélgica).

Figura 4 – Visão lateral do conjunto formado pelas placas de vidro, cimento endodôntico e peso de metal



Fonte: Tanomaru-Filho et al.[7,p.797].

Figura 5AB- Ilustração das áreas avaliadas no programa CTAn



A – Preenchimento volumétrico da cavidade central (PVC) e B – preenchimento volumétrico da cavidade lateral (PVL) 2 mm além da área central.

Fonte: Tanomaru-Filho et al.[7,p.798].

Subprojeto 4: Avaliação da alteração volumétrica, solubilidade e presença de vazios para biomateriais de silicato de cálcio

Foram avaliados solubilidade, alteração volumétrica e presença de vazios nos cimentos biocerâmicos TotalFill BC Sealer (FKG Dentaire SA, La Chaux-de-Fonds, Switzerland), Sealer Plus BC (MK Life, Porto Alegre, RS, Brasil) e Bio-C Sealer (Angelus, Londrina, PR, Brasil), em comparação ao cimento à base de resina epóxi considerado padrão ouro (AH Plus).

Solubilidade

Foram confeccionados corpos de prova medindo 7,75 mm de diâmetro e 1,5 mm de altura (n=6), com base em estudo prévio[3]. Cada molde foi preenchido com o cimento a ser avaliado e posicionado sobre lamínula de vidro recoberta por uma película de papel celofane. Um fio de nylon impermeável foi colocado no interior do material e outra placa de vidro, também coberta com uma película de celofane, foi colocada sobre o molde e pressionada manualmente, de tal maneira que as placas tocassem a superfície do molde uniformemente. O conjunto foi armazenado em estufa com temperatura de 37°C e 95% de umidade até a completa presa do material, um período correspondente a três vezes o tempo de presa. Os corpos de prova foram removidos dos moldes, colocados em dessecador a vácuo, e a massa foi mensurada em balança de precisão até sua estabilização. Na sequência, os espécimes foram suspensos por meio da fixação dos fios de nylon no interior de recipientes plásticos com tampa contendo 7,5 mL de água destilada e deionizada, tendo o cuidado de evitar qualquer contato entre o material e a superfície interna do recipiente. Os recipientes permaneceram em estufa a 37°C pelo período de 7 dias, quando os corpos de prova foram removidos da água destilada, lavados com água destilada e deionizada, secos com papel absorvente e colocados novamente em desumidificador até obter estabilidade da massa final. O teste foi realizado mantendo os corpos de prova imersos também em PBS pelo mesmo período. A variação de massa foi expressa em porcentagem da massa original.

Teste de alteração volumétrica e presença de vazios em micro-CT utilizando modelo de teste de solubilidade

A metodologia em micro-CT foi realizada de maneira similar ao descrito para o subprojeto 2, utilizando corpos de prova de 7,75 mm de diâmetro e 1,5 mm de altura (n=6), de acordo com estudo prévio[8]. Além da comparação entre os volumes inicias e após imersão em água ou PBS, o percentual de vazios no interior dos cimentos também foi quantificado por meio do software CTAn.

Subprojeto 5: Avaliação de materiais endodônticos em diferentes resoluções

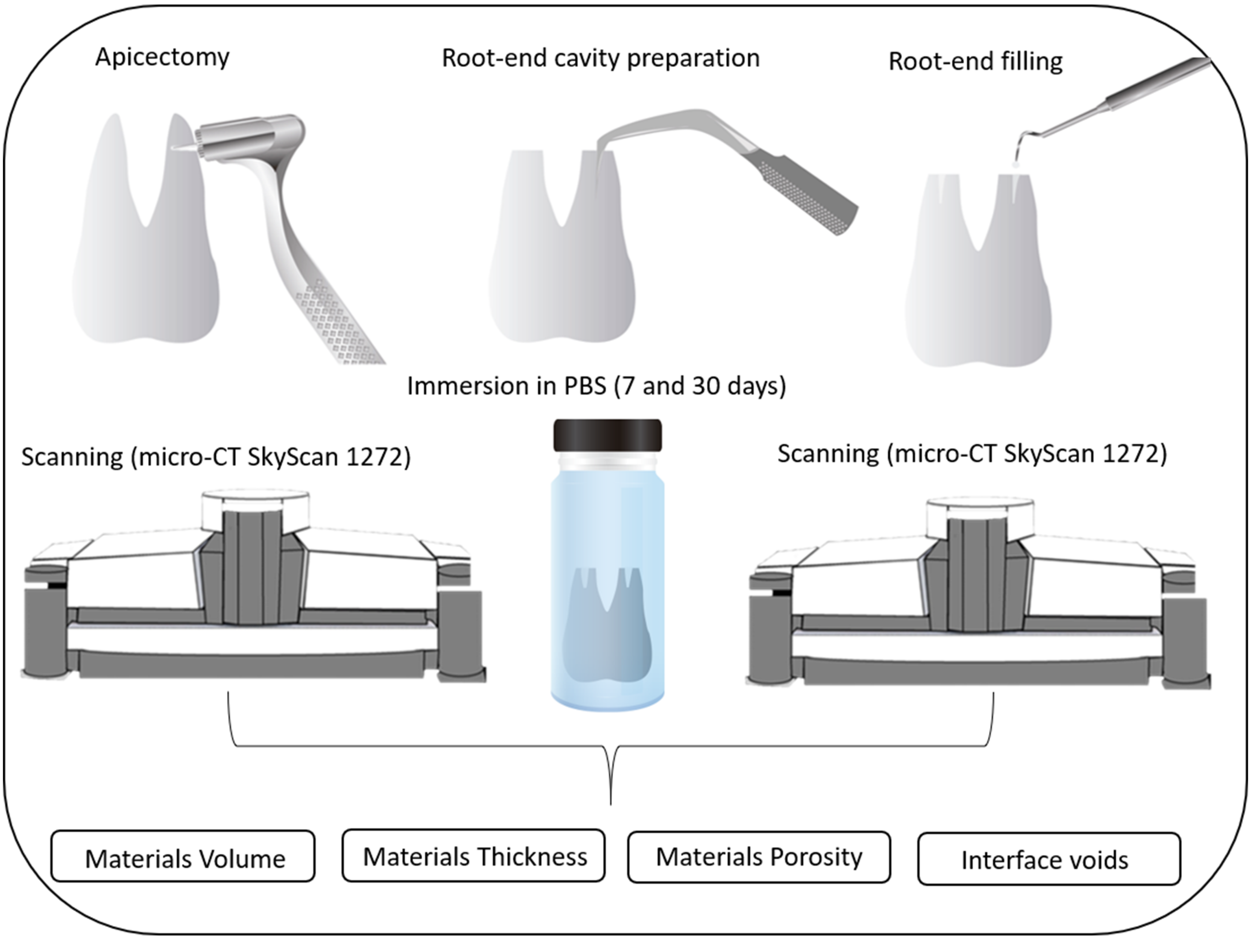
Este subprojeto foi dividido em três partes e conduzido por meio de Bolsa BEPE (Processo 2017/22481-1).

Preparo das amostras

Foram utilizados 12 pré-molares superiores com duas raízes separadas (24 raízes). Este estudo foi aprovado pela Faculdade de Odontologia de Araraquara, Comitê de Ética em Pesquisa da UNESP (n° CAAE: 9779617.5.0000.5416) (Anexo A).

Foi realizada apicectomia das raízes e retropreparo de 3 milímetros de profundidade utilizando a ponta ultrassônica T1F-R (CVD-Vale, São José dos Campos, SP, Brazil). As cavidades foram preenchidas com MTA, Biodentine e IRM. As amostras foram mantidas em estufa a 37° C e 95% de umidade por três vezes o tempo de presa.

Figura 6 – Esquema ilustrando o processo de preparo e avaliação dos espécimes no subprojeto 5



Após a seleção de pré-molares superiores com duas raízes separadas, foi realizada apicectomia, retropreparo e retrobturação dos dentes selecionados, além do escaneamento em micro-CT após a presa e após a imersão dos espécimes em PBS por 7 e 30 dias.

Fonte: Torres et al.[9,p.2].

Aquisição e segmentação das imagens

As amostras foram escaneadas com o sistema SkyScan 1272 (SkyScan, Bruker-microCT, Kontich, Bélgica), seguindo os parâmetros: 5 µm de tamanho de voxel, 100 kVp, 100 µA e 0,11 mm de filtro de cobre. Após o escaneamento inicial as amostras foram mantidas imersas em PBS (1x) e armazenadas em estufa a 37° C entre os intervalos experimentais de 7 e 30 dias. Após esses períodos, novos escaneamentos foram realizados usando os mesmos parâmetros.

As imagens foram reconstruídas usando o software NRecon (V1.6.10.4; Bruker-MicroCT, Kontich, Bélgica). Os conjuntos de dados foram exportados usando o formato de arquivo DICOM.

Os conjuntos de dados registrados foram importados para segmentação em uma ferramenta dedicada desenvolvida no MeVisLab (MeVis Research, Bremen, Alemanha) e validados para segmentação precisa do espaço do dente/canal radicular. Na parte 2 deste subprojeto, as imagens reconstruídas também foram segmentadas diferenciando material e dentina utilizando um limiar semi-automático no software CTAn (V1.15.4.0; Bruker-MicroCT, Kontich, Bélgica). Em ambos os softwares a segmentação foi realizada a 5 µm, e após redimensionar as imagens para 10 e 20 µm.

Alteração volumétrica e morfológica

Após a segmentação, os volumes dos materiais foram registrados. Análises morfológicas foram realizadas no software 3-matic (Materialis, Leuven, Bélgica). Além disso, foi realizada a segmentação de poros e, em seguida, a porosidade total dos cimentos foi calculada como uma porcentagem do volume total (Amira, FEI Visualization Sciences Group). Na parte 2 deste subprojeto o volume e porosidade dos materiais também foi avaliado pelo software CTAn e em todos os softwares as imagens foram avaliadas a 5, 10 e 20 µm.

Análise de interface

As diferenças nas porcentagens de vazios na interface entre a superfície dentinária das paredes do canal radicular e os materiais retrobturadores foram avaliadas com base no método descrito em estudo prévio[10], de forma que vazios com tamanho a partir de 5 µm foram detectados. Modelos 3D dos vazios foram criados usando o software CTAn e exportados para o programa 3-matic para analisar a espessura desses vazios. Na parte 2 deste subprojeto a interface também foi avaliada a 10 e 20 µm.

Análise dos resultados

Para todos os subprojetos, os resultados obtidos foram submetidos a um teste de normalidade, e posteriormente submetidos aos testes estatísticos ANOVA, Tukey e Teste-T, com 5% de significância.

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ANEXO A – COMITÊ DE ÉTICA

UNESP - FACULDADE DE ODONTOLOGIA - CAMPUS ARARAQUARA

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Efeito do escaneamento e da análise em microtomografia na avaliação das propriedades de cimentos reparadores

Pesquisador: Mario Tanomaru Filho

Área Temática:

Versão: 2

CAAE: 79779617.5.0000.5416

Instituição Proponente: Faculdade de Odontologia de Araraquara - UNESP

Patrocinador Principal: FUNDACAO DE AMPARO A PESQUISA DO ESTADO DE SAO PAULO

DADOS DA NOTIFICAÇÃO

Tipo de Notificação: Envio de Relatório Final

Detalhe:

Justificativa: O Relatório Final apresentado descreve o desenvolvimento do Projeto durante o

Data do Envio: 06/12/2018

Situação da Notificação: Parecer Consubstanciado Emitido

DADOS DO PARECER

Número do Parecer: 3.145.160

UNESP - FACULDADE DE ODONTOLOGIA - CAMPUS ARARAQUARA

Continuação do Parecer: 3.145.160

Tipo Documento	Arquivo	Postagem	Autor	Situação
Envio de Relatório Final	RELATORIO_FINAL.pdf	06/12/2018 11:13:53	Mario Tanomaru Filho	Postado

Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Não

ARARAQUARA, 13 de Fevereiro de 2019

Assinado por:
Andréa Gonçalves
(Coordenador(a))

ANEXO B – POLÍTICAS DE COPYRIGHT E DE AUTOARQUIVO DE EDITORES EM “JOURNAL AND PUBLISHER POLICIES ON AUTHOR SELF-ARCHIVING (EPRINTS/ROMEO VERSION)”: JOURNAL OF APPLIED ORAL SCIENCE*

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Pesquisa - Políticas de copyright e de auto-arquivo de editores

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Pesquisa - Políticas de copyright e de auto-arquivo de editores

Uma revista encontrada ao pesquisar: **international endodontic journal**

Revista: International Endodontic Journal (ISSN: 0143-2885, ESSN: 1365-2591)

RoMEO: This is a RoMEO **yellow** journal

Paid OA: Uma taxa de acesso aberto **está disponível** para esta revista.

Versão preprint do autor: O autor **pode** arquivar a versão preprint (i.e. antes do peer-review)

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- 12 meses de embargo

versão/PDF do editor: O autor **não pode** arquivar a versão/PDF do editor

Condições gerais:

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- Tem de ser mencionada a fonte publicada com citação
- Must link to publisher version with set statement (see policy)
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ANEXO G – POLÍTICAS DE COPYRIGHT E DE AUTOARQUIVO DE EDITORES EM "JOURNAL AND PUBLISHER POLICIES ON AUTHOR SELF-ARCHIVING (EPRINTS/ROMEO VERSION)": JOURNAL OF MATERIALS SCIENCE:MATERIALS IN MEDICINE

SHERPA/RoMEO . . . *opening access to research*

Pesquisa - Políticas de copyright e de auto-arquivo de editores

Uma revista encontrada ao pesquisar: **journal of materials science materials in medicine**

Revista: Journal of Materials Science: Materials in Medicine (ISSN: 0957-4530, ESSN: 1573-4838)

RoMEO: This is a RoMEO **green** journal

Paid OA: Uma taxa de acesso aberto **está disponível** para esta revista.

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- Author's post-print on author's personal website immediately
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- A versão/PDF do editor não pode ser utilizada
- Tem de ser mencionada a fonte de publicação
- Tem de ser feita uma ligação para a versão do editor
- Set phrase to accompany link to published version (see policy)
- Os artigos de algumas revistas podem ficar em Acesso Aberto mediante pagamento adicional

ANEXO H – POLÍTICAS DE COPYRIGHT E DE AUTOARQUIVO DE EDITORES EM "JOURNAL AND PUBLISHER POLICIES ON AUTHOR SELF-ARCHIVING (EPRINTS/ROMEO VERSION)": IMAGING SCIENCE IN DENTISTRY

SHERPA/RoMEO . . . *opening access to research*

Pesquisa - Políticas de copyright e de auto-arquivo de editores

Uma revista encontrada ao pesquisar: **imaging science in dentistry**

Revista:	**Imaging Science in Dentistry** (ISSN: 2233-7822, ESSN: 2233-7830)
RoMEO:	This is a RoMEO **ungraded** journal
	- As políticas deste editor não foram verificadas por RoMEO - Se necessário, por favor, entre em contacto com o editor para informações adicionais
Actualizado:	Please contact us if you wish to suggest adding this publisher properly to RoMEO
Ligação para esta página:	http://sherpa.ac.uk/romeo/issn/2233-7822/pt/
Publicado por:	**Korean Academy of Oral and Maxillofacial Radiology** (according to *Zetoc*)

Korean Medical Journal Information

KAMJE KoreaMed Synapse KoMCI

Journal Title:	Imaging Science in Dentistry
Journal Abbreviation:	Imaging Sci Dent
Acronym:	ISD
Publication Date:	Vol. 41, no. 1 (2011) -
Frequency:	Quarterly
Publisher :	Korean Academy of Oral and Maxillofacial Radiology
Language :	English
pISSN:	2233-7822
eISSN:	2233-7830
DOI Prefix:	10.5624/isd
Continues:	Korean Journal of Oral and Maxillofacial Radiology = 대한구강악안면방사선학회지
Broad Subject Term(s):	Dentistry Radiology
MeSH (NLM):	Radiography, Dental Stomatognathic Diseases/radiography
SC (SCI):	Dentistry, Oral Surgery & Medicine
Open Access:	OA-nc (https://creativecommons.org/licenses/by-nc/3.0/)
Electronic Links:	https://isdent.org/ https://synapse.koreamed.org/LinkX.php?code=2080ISD https://koreamed.org/volumes/2080 https://www.ncbi.nlm.nih.gov/nlmcatalog/101559249
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ANEXO I – POLÍTICAS DE COPYRIGHT E DE AUTOARQUIVO DE EDITORES EM "JOURNAL AND PUBLISHER POLICIES ON AUTHOR SELF-ARCHIVING (EPRINTS/ROMEO VERSION)": MICROSCOPY RESEARCH AND TECHNIQUE*

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Início • Pesquisa • Revistas • Editores

Pesquisa - Políticas de copyright e de auto-arquivo de editores

English |

This version of Sherpa Romeo will be decommissioned on Tuesday 14 April 2020. Our new version is available at https://v2.sherpa.ac.uk/romeo.

Uma revista encontrada ao pesquisar: **microscopy research and technique**

Revista:	Microscopy Research and Technique (ISSN: 1059-910X, ESSN: 1097-0029)
RoMEO:	This is a RoMEO **yellow** journal
Paid OA:	Uma taxa de acesso aberto **está disponível** para esta revista.
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