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

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Características físico-químicas do clínquer com diferentes tamanhos de partículas e de cimentos reparadores e obturadores à base de silicato de cálcio

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Tese apresentada à Universidade Estadual Paulista ¨Júlio de Mesquita Filho¨ - UNESP, Faculdade de Odontologia de Araraquara, para obtenção do título de Doutor em Odontologia, na Área de Endodontia

Orientador: Prof. Dr. Mário Tanomaru Filho

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Comissão julgadora

Tese para obtenção de grau de Doutor em Odontologia

Presidente e orientador: Prof. Dr. Mário Tanomaru Filho

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Araraquara, 25 de março de 2024.

DADOS CURRICULARES

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“Nunca pare, não se detenha até que o que você sonhou se torne realidade. Mas primeiro ACREDITE”
Daniel Habif*

* Habif D. Inquebrantables. Mexico: HarperCollins México; 2019.

Tavares KIMC. Características físico-químicas do clínquer com diferentes tamanhos de partículas e de cimentos reparadores e obturadores à base de silicato de cálcio [tese de doutorado]. Araraquara: Faculdade de Odontologia da UNESP; 2024.

RESUMO

Novas formulações de cimentos à base de silicato de cálcio são desenvolvidas. Avaliação de propriedades físico-químicas do clínquer (CL) Angelus (Londrina, PR, Brasil) e de cimentos reparadores e obturadores biocerâmicos empregando diferentes metodologias foram realizadas. Este estudo foi dividido em três publicações: **Publicação 1:** Avaliou o efeito do tamanho de partícula (2 a 30 µm e <2 µm) associado ao tungstato de cálcio ($CaWO_4$) e manipulado com água destilada (AD) ou líquido com aditivos (LA) nas propriedades físicas do CL, em comparação ao MTA Branco e MTA Repair HP. CL 2 a 30 µm apresentou menor tempo de presa (TP) inicial e maior TP final quando manipulado com LA ($p<0,05$). A adição de $CaWO_4$ aumentou a radiopacidade do CL para ambas partículas ($p<0,05$), porém não afetou TP, solubilidade e pH ($p>0,05$). Todos os materiais apresentaram pH alcalino ($p>0,05$), principalmente nas 24 horas e 7 dias. Quanto à solubilidade, todos os grupos apresentaram ganho de massa, exceto MTA em AD ($p<0,05$). CL 2 a 30 µm + $CaWO_4$ apresentaram maior ganho de massa e maior radiopacidade quando manipulados com LA que com AD ($p<0,05$). CL<2 µm manipulado com LA proporciona menor TP final. Ambos tamanhos de partícula influenciam positivamente na capacidade de alcalinização do CL, principalmente nos períodos iniciais. **Publicação 2:** Investigou o efeito da imersão em ácido butírico (AB, pH 4,1) ou PBS (pH 7,0) nas propriedades do CL com tamanho de partícula de 2 a 30 µm ou < 2 µm associado ao óxido de zircônio e manipulado com AD ou LA em comparação com Bio-C Repair (BCR) e Biodentine (BIO).Todos os materiais apresentaram alterações volumétricas semelhantes ao BCR e BIO ($p>0,05$). AB mostrou maior aumento da porosidade (aproximadamente 8%) do que PBS (aproximadamente 2%), exceto para CL2 a 30 µm com LA ($p<0,05$). A imersão em AB por 28 dias aumentou a porosidade e falhas na interface quando comparado ao baseline ($p<0,05$). CL 2 a 30 µm com AD apresentou maior porosidade e falhas na interface que os demais grupos ($p<0,05$). Análise MEV revelou formação de hidroxiapatita na superfície do material em PBS e perda estrutural em AB. pH ácido prejudica a interface material/dentina, porosidade e promove alterações dimensionais dos materiais biocerâmicos. AD sem aditivos aumenta a porosidade, falhas de interface e perda de volume do CL. **Publicação 3:** Avaliou o efeito da imersão em ambiente ácido ou neutro no comportamento físico do Bio-C Sealer (BCS) ou AH Plus Jet (AHPJ) após obturação de canais ovais empregando diferentes espessuras de cimento. Alterações volumétricas e porcentagem de vazios e falhas na interface de BCS e AHPJ foram observadas em ambiente ácido após 30 dias ($p<0,05$). Ambos materiais apresentaram alto percentual de vazios e perda volumétrica na maior espessura do cimento ($p< 0,05$). Baseline apresentou menor percentual de vazios e falhas na interface quando comparado aos períodos experimentais ($p<0,05$). Meio ácido prejudica as propriedades dos cimentos após 30 dias. A maior espessura do cimento afeta negativamente a alteração volumétrica e presença de vazios nos canais ovais.

Palavras-chave: Endodontia. Materiais dentários. Microtomografia por Raio-X. Fenômenos físicos.

Tavares KIMC. Physicochemical characteristics of clinker with different particle sizes besides root repair cements and root canal sealers based on calcium silicate [tese de doutorado]. Araraquara: Faculdade de Odontologia da UNESP; 2024.

ABSTRACT

New cement formulations based on calcium silicate are developed. Assessment of physical-chemical properties of Clinker Angelus (CL) (Londrina, PR, Brazil) and bioceramic repair and filling materials using different methodologies was performed. This study was divided into three publications: **Publication 1**: Evaluated the effect of particle size (2 to 30 µm and <2 µm) associated with calcium tungstate ($CaWO_4$) and manipulated with distilled water (DW) or liquid with additives (LA) on the physical properties of CL, compared with White MTA and MTA Repair HP. CL 2 to 30 µm showed shorter initial ST and higher final ST when manipulated with LA ($p<0.05$). The addition of $CaWO_4$ increased the CL radiopacity for both particle sizes ($p<0.05$), but did not influence ST, solubility, and pH ($p>0.05$). All materials presented alkaline pH ($p>0.05$), especially in 24 hours and 7 days. Regarding the solubility, all groups showed mass gain, except for MTA in DW ($p<0.05$). CL 2 to 30 µm + $CaWO_4$ showed greater mass gain and higher radiopacity when manipulated with LA than DW ($p<0.05$). **Conclusions:** CL<2 µm manipulated with LA provides shorter final ST. Both particle sizes positively influence the alkalization capacity of CL, especially in the initial periods. **Publication 2:** Investigated the effect of immersion in butyric acid (BA, pH 4.1) or PBS (pH 7.0) on the properties of CL with a particle size of 2 to 30 µm or < 2 µm associated with zirconium oxide and manipulated with DW or LA compared to Bio-C Repair (BCR) and Biodentine (BIO). All groups exhibited volumetric changes similar to BCR and BIO ($p>0.05$). BA showed greater increase in porosity (approximately 8%) than PBS (approximately 2%), except for CL2 to 30 µm with LA ($p<0.05$). Immersion in BA for 28 days increased porosity and the presence of gaps in the material/dentin interface for all groups when compared to baseline ($p<0.05$). CL 2 to 30 µm with DW showed greater porosity and gaps in the interface compared to the other groups ($p<0.05$). SEM analysis revealed that all groups showed hydroxyapatite formation on the material surface in PBS and structural loss in BA. Acidic pH damages the material/dentin interface, porosity, and promotes dimensional changes for bioceramics. DW without additives increases porosity, interface gaps, and volume loss of CL. **Publication 3:** Evaluated the effect of immersion in an acidic or neutral environment on the physical behavior of Bio-C Sealer (BCS) or AH Plus Jet (AHPJ) after filling oval canals using different cement thicknesses. Significant volumetric changes and percentage of voids and gaps in the interface of BCS and AHPJ were observed in an acidic environment after 30 days ($p<0.05$). Both materials showed a high percentage of voids and volumetric loss in the greater sealer thickness ($p< 0.05$). The baseline showed a lower percentage of voids and gaps in the interface when compared to the experimental periods ($p<0.05$). Acidic environment impairs the properties of sealers after 30 days. The greater thickness of the sealer negatively affects the volumetric change and the presence of voids in oval canals.

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

SUMÁRIO

1 INTRODUÇÃO

Cimentos reparadores e obturadores à base de silicato de cálcio são amplamente empregados na Endodontia[1-4]. Mineral Trióxido Agregado (MTA) é composto por silicatos de cálcio e óxido de bismuto como agente radiopacificador[5]. MTA apresenta limitações como consistência arenosa, possibilidade de manchamento dentário e da presença de metais pesados[6,7].

Diferentes radiopacificadores podem ser utilizados nos cimentos de silicatos de cálcio, no intuito de superar a descoloração dentinária atribuída a presença do óxido de bismuto[6,8,9]. Dentre eles, o tungstato de cálcio ($CaWO_4$) e o óxido de zircônio (ZrO_2) merecem destaque. $CaWO_4$ ou ZrO_2 quando associados a cimentos à base de silicato tricálcico puro promovem aumento da resistência à compressão sem alterar o tempo de presa final[8], além de proporcionar ação antimicrobiana[10,11], biocompatibilidade[12], citocompatibilidade[12,13], pH alcalino, liberação de íons cálcio[14], bioatividade[15] e propriedades físicas adequadas[11,16]. Além disso, o emprego de $CaWO_4$ ou ZrO_2 como agentes radiopacificadores não promovem alterações cromáticas quando em contato com o hipoclorito de sódio[17].

Os cimentos biocerâmicos da Angelus (Angelus® Indústria de Produtos Odontológicos S/A) são produzidos a partir do clínquer confeccionados com diferentes tamanhos de partículas (2 a 30 µm ou inferior a 2 µm). De acordo com o fabricante, as matérias-primas puras utilizadas como carbonato de cálcio, óxido de alumínio e dióxido de silício são submetidas aos processos de homogeneização, secagem e calcinação para obtenção do clínquer, composto por silicato tricálcico, silicato dicálcico, aluminato tricálcico e óxido de cálcio. Em seguida, esse pó é submetido a um processo especial de moagem, responsável pela formação das finas partículas dos grãos do material, que posteriormente são associadas ao agente radiopacificador. A adição do tungstato de cálcio e manipulação com diferentes líquidos não influenciam nas propriedades físico-químicas e biológicas do Clínquer de 2 a 30 µm[18]. Até o momento, não existe na literatura, estudos que avaliem o impacto do tamanho de partícula após a associação ao radiopacificador e manipulação com diferentes líquidos nas propriedades físicas do Clínquer Angelus.

MTA Repair HP (MTAHP, Angelus, Londrina, PR, Brasil) é um material reparador à base de silicato de cálcio pó-líquido com aditivos no líquido para melhor

manipulação e posterior inserção clínica[19]. MTAHP apresenta biocompatibilidade e capacidade de induzir mineralização[19-23], além do tempo de presa curto[21,24,25], escoamento e radiopacidade de acordo com as normas ISO 6876-2012[20,26], resistência à compressão[27,28], pH alcalino e atividade antimicrobiana[11,29]. Solubilidade baixa ou valores acima daqueles recomendados pelas diretrizes ISO foram relatados para MTAHP [11,20,24]. Por outro lado, MTAHP mostrou baixa alteração volumétrica quando imerso em água destilada[30], porém maior porosidade e presença de falhas na interface material/dentina também foram apontadas para este material usando modelo de tubo de dentina implantado no tecido subcutâneo de ratos[23]. No entanto, ainda não existem estudos *in vitro* que avaliem a interface dentinária e comportamento volumétrico deste material após desafio ácido usando modelos de tubos de dentina bovina.

Biodentine (BIO, Septodont, Saint Maur des Fossés, França) apresenta em sua composição silicato tricálcico puro e óxido de zircônio como radiopacificador. BIO apresenta propriedades mecânicas superiores ao MTA, além de menor tempo de presa e maior estabilidade de cor[31], com adequada biocompatibilidade e bioatividade[32]. No entanto, maior solubilidade após imersão em água destilada é descrita para BIO em comparação ao MTA Branco (Angelus, Londrina, PR, Brasil) e cimento de óxido de zinco e eugenol (S.S.White Art. Dent. Ltda., Rio de Janeiro, RJ, Brasil) [33]. Por outro lado, a imersão de BIO em ácido butírico proporcionou alteração volumétrica[34], sem diminuir seus valores de resistência à compressão[35].

Bio-C Repair (BCR, Angelus, Londrina, PR, Brasil) é um material reparador pronto para uso à base de silicato de cálcio, composto por óxido de cálcio, óxido de zircônio, óxido de ferro, dióxido de silício e agente de dispersão. BCR apresenta biocompatibilidade e potencial biativo[23,36-38], além de citocompatibilidade[39-42]. BCR apresenta maior capacidade de preenchimento e menor alteração volumétrica que MTAHP[30,38], além de adequada radiopacidade, pH alcalino e valores de solubilidade inferiores a 3%[38,41]. Baixa porosidade e adequada capacidade de selamento na interface material/dentina também são descritas para este material[23]. No entanto, BCR mostrou maior percentual de vazios em comparação ao MTA Branco[43]. Todavia, não existem estudos avaliando a interação deste material com a dentina após imersão em soluções que mimetizem diferentes condições de pH.

O tamanho de partícula e o tipo de líquido empregado influenciam as propriedades dos materiais à base de silicato de cálcio[44-47]. A presença de partículas menores em cimentos reparadores hidráulicos melhora a capacidade seladora, diminui o tempo de presa e a espessura de película, além de permitir maior escoamento e otimizar o potencial antimicrobiano[48-50]. Em contrapartida, o aumento do tamanho das partículas provou afetar adversamente o tempo de presa, pH e a resistência à compressão do MTA[51-53]. Além disso, quando o pó do material ou seu agente radiopacificador apresentam partículas menores (0.8 µm - 1,5 µm), maior área de superfície é estabelecida, melhorando as propriedades físico-químicas[15,49,54], mecânicas[50] e biológicas dos materiais à base de silicato de cálcio[48,54,55].

Camilleri et al.[56] (2013) afirmaram que o clínquer do MTA Angelus proveniente da construção civil era sinterizado incompletamente, apresentando uma menor quantidade de silicato tricálcico em sua composição quando comparado ao Biodentine e um cimento experimental (80% silicato tricálcico e 20% óxido de zircônio). Atualmente, segundo informações do fabricante, o clínquer do MTA Angelus é fabricado com matéria prima constituída de compostos puros que são sinterizados em forno elétrico apropriado (Angelus® Indústria de Produtos Odontológicos S/A).

O comportamento dos materiais pode ser afetado pela variabilidade do potencial de hidrogênio (pH)[34,57-64]. pH proporcionado pelo uso de fluidos corporais simulados diminui a solubilidade e alteração volumétrica de materiais à base de silicatos de cálcio[61-62]. Além disso, sua similaridade com um ambiente clínico permite avaliar adequadamente o comportamento físico de materiais hidrofílicos[63,64]. Em contrapartida, um pH ácido, que pode ocorrer em lesões periapicais pode alterar a composição química e o processo de hidratação de materiais biocerâmicos[57], exercendo efeito direto em suas propriedades físicas[34,58-60]. No entanto, o comportamento de cimentos biocerâmicos dentro de um ambiente ácido não está totalmente elucidado na literatura.

A obturação dos canais radiculares é realizada usando cones de guta-percha e cimento endodôntico[65], visando o selamento tridimensional[66]. Bio-C Sealer (BCS, Angelus, Londrina, PR, Brasil) é um cimento pronto para uso, composto por silicatos de cálcio, aluminato de cálcio, óxido de cálcio, óxido de zircônio, óxido de ferro, dióxido de silício e agente de dispersão. BCS apresenta adequada radiopacidade, tempo de presa, escoamento, pH alcalino e baixa alteração volumétrica revelada por análises

em microtomografia computadorizada[67], além de demonstrar capacidade de preenchimento similar ao AH Plus[68] e baixa presença de gaps marginais quando comparado ao AH Plus Jet[69]. No entanto, este cimento apresenta valores de solubilidade acima daqueles preconizados pelas normas ISO[63,68,70]. BCS apresenta biocompatibilidade[71], potencial bioativo[72] e capacidade antibacteriana[73], além de maior citocompatibilidade e capacidade de mineralização quando comparado ao AH Plus[71].

AH Plus (Dentsply DeTrey GmbH, Konstanz, Alemanha) é um cimento à base de resina epóxi comumente utilizado como padrão de comparação para investigação de novos cimentos[39,64,68,74] devido às suas excelentes propriedades físico-químicas[70,74,75]. Atualmente, este cimento encontra-se comercializado em uma seringa com mistura automática (AH Plus Jet)[78,79].

A recontaminação microbiana está diretamente relacionada com a presença de vazios no interior do material obturador[76]. Uma fina camada de cimento é desejável entre os cones de guta-percha e a parede do canal radicular, pois diminui a possibilidade de infiltração[2]. A técnica de cone único é de baixo custo e de fácil execução, sendo frequentemente associada ao uso dos cimentos biocerâmicos[2]. No entanto, maior percentual de falhas na interface material/guta-percha para o cimento EndoSequence BC Sealer (Brasseler, Savannah, GA,USA) em comparação ao AH Plus foi observado quando empregado a técnica de cone único[74].

Diversos métodos são utilizados para avaliação dos materiais à base de silicato de cálcio[7,11,33,64]. Testes empregando microtomografia computadorizada (micro-CT) e microscópio eletrônico de varredura (MEV) representam alternativas metodológicas importantes[33,64,67-69,78,79]. Micro-CT é considerada um método 3D alternativo e preciso para avaliação da capacidade de preenchimento de materiais, identificado vazios no interior do material obturador[1,30,43,64,66,68,69,74,80] e na interface com a dentina[23,43,69,74], além de possibilitar o estudo de diversas propriedades físicas, como a alteração de volume[30,34,38,46,58,60,62,64,67] e a porosidade[23,33] de materiais após imersão em diferentes meios. Por outro lado, MEV permite analisar as características microestruturais dos materiais[80,81], acrescentando informações adicionais aos testes convencionais[33] e análises por meio de micro-CT[82].

Levando em consideração os importantes avanços dos materiais à base de silicato de cálcio e a necessidade de investigações que apoiem o uso clínico, este projeto visa avaliar as propriedades físico-químicas do clínquer Angelus, além da investigação das propriedades físico-químicas de cimentos reparadores e obturadores usando diferentes abordagens metodológicas. Para o estabelecimento de comparações, cimentos reparadores e endodônticos disponíveis comercialmente foram empregados.

2 PROPOSIÇÃO

Avaliou as propriedades físico-químicas do clínquer da Angelus com diferentes tamanhos de partículas (2 a 30 µm e <2 µm), antes e após adição de radiopacificador e manipulado com água destilada ou líquido com aditivos, além da análise de cimentos reparadores e endodônticos, por meio de metodologias convencionais, micro-CT e MEV.

2.1 Objetivos Específicos

Publicação 1 - Investigou o Clínquer Angelus com diferentes tamanhos de partículas (2 a 30 µm e <2 µm) associado ao tungstato de cálcio como radiopacificador e manipulado com água destilada ou líquido com aditivos quanto a seu tempo de presa, radiopacidade, solubilidade e pH, em comparação com MTA Branco e MTA Repair HP.

Publicação 2 - Avaliou em micro-CT, o efeito do pH na interface material/dentina, porosidade e alteração volumétrica, além de análises da superfície por MEV do clínquer Angelus (2 a 30 µm ou <2 µm) associado ao radiopacificador óxido de zircônio, manipulado com água destilada ou líquido com aditivos antes e após imersão em ácido butírico ou PBS em diferentes períodos, em comparação com Bio-C Repair e Biodentine.

Publicação 3 - Investigou em micro-CT, o efeito do pH e da espessura de cimento na interface cimento/dentina, alteração volumétrica e porcentagem de vazios em canais radiculares ovais após obturação com cimentos Bio-C Sealer ou AH Plus Jet, antes e após imersão em ácido butírico ou PBS em diferentes períodos.

3 PUBLICAÇÕES

Está pesquisa resultou nas publicações apresentadas a seguir

3.1 Publicação 1*

Influence of particle size, radiopacifier, and liquids on the physical properties of Bioceramic Clinker Angelus: An *In-Vitro* Investigation

ABSTRACT

Introduction: To assess the effect of the particle size (2 to 30 µm and <2 µm), the addition of calcium tungstate ($CaWO_4$) as a radiopacifier and manipulation with distilled water (DW) or liquid with additives (LA) on the physical properties of Clinker Angelus (CL), compared with White MTA and MTA Repair HP. **Methods:** Setting time (ST) and radiopacity were evaluated according to ISO 6876/2012, and solubility was evaluated after immersion in DW or PBS after 7 days. The pH was measured using a digital pH meter in 1, 3, 7, 14, 21, and 28 days. ANOVA and Tukey and unpaired *t*-test were performed (α = 0.05). **Results:** CL 2 to 30 µm showed shorter initial ST and higher final ST when manipulated with LA ($P < .05$). The addition of $CaWO_4$ increased the CL radiopacity for both particle sizes ($P < .05$), but did not influence ST, solubility, and pH ($P > .05$). All materials presented alkaline pH ($P > .05$), especially in 24 hours and 7 days. Regarding the solubility, all groups showed mass gain, except for White MTA in DW ($P < .05$). CL 2 to 30 µm + $CaWO_4$ showed greater mass gain and higher radiopacity when manipulated with LA than DW ($P < .05$). **Conclusions:** CL<2 µm manipulated with LA provides shorter final ST. Both particle sizes positively influence the alkalization capacity of CL, especially in the initial periods. The addition of calcium tungstate and manipulation with LA showed adequate physical properties for CL.

KEY WORDS Calcium silicate; Dental Materials; Endodontics; Physical process

* Artigo escrito nas normas do periódico *Journal of Endodontics*, ao qual foi submetido.

INTRODUCTION

Materials based on calcium silicates, such as Mineral Trioxide Aggregate (MTA) from Angelus (Angelus® Indústria de Produtos Odontológicas S/A, Londrina, PR, Brazil) are made with raw materials manufactured from pure compounds using calcium carbonate, aluminum oxide and silicon dioxide. Subsequently, the use of an electric kiln is used to sinter the powder called Clinker with different particle sizes (2 to 30 µm or < 2 µm), providing a lower risk of contamination (1). Next, the Clinker is subjected to a grinding process, responsible for forming fine-grain particles of the material, which are subsequently associated with the radiopacifier agent. The association of pure tricalcium silicate with calcium tungstate as a radiopacifier provides excellent biological, antimicrobial (2), physical (2, 3), and mechanical properties (4).

The particle size can influence the performance of endodontic materials (5-9). In the presence of larger particles, the hydration of the material is incomplete, compromising the setting time, alkalization capacity, compressive strength, and interaction of the material with dentin (5, 7). On the other hand, smaller particles provide shorter setting time, antibacterial activity, and adequate sealing (8, 9). Furthermore, when the material or radiopacifier powder has smaller particles (1.5 µm or 0.8 µm), a greater surface area is established, favoring the physicochemical, mechanical, and biological properties of calcium silicate-based cements (5, 7-10).

Material handling involves mixing the powder with liquid. The establishment of an adequate proportion allows to obtain a homogeneous mixture, favoring the physicochemical properties of hydraulic materials (11). A high amount of water impairs the dimensional stability of MTA (12). Furthermore, the incorporation of organic agents in distilled water aims to improve the handling and insertion of the material (13). However, greater solubility, porosity, and gaps in the material/dentin interface have been reported when using liquid with additives (13, 14).

Considering that the repair materials are formulated from Clinker powder associated with a radiopacifier and manipulated with liquids, the aim of this study was to evaluate the effect of the particle size (2 to 30 µm and < 2 µm), the addition of calcium tungstate as a radiopacifier agent and manipulation with distilled water or liquid with additives on the physical properties of Clinker Angelus, compared to White MTA and MTA Repair HP (Angelus). The null hypothesis is that there would be no difference between the physical properties of the materials evaluated.

MATERIAL AND METHODS

The materials with their respective manufacturers, compositions, and proportions are described in Table 1. Pilot studies were performed to adequately determine the powder/liquid proportion for each experimental group.

Table 1 – Experimental groups, material with their manufacturers, composition, and proportion

Groups	Material and Manufacturer	Composition	Proportion
CL 2 to 30 µm + DW or LA	Clinker 2 to 30 µm Angelus, Londrina, PR, Brazil Lot: 61186	Powder: tricalcium silicate, dicalcium silicate, tricalcium aluminate and calcium oxide Liquid: distilled water or liquid with additives (water and polyvinylpyrrolidone)	1g powder : 300 µL distilled water 1g powder : 300 µL liquid with additives
CL < 2 µm + DW or LA	Clinker < 2 µm Angelus, Londrina, PR, Brazil Lot: 60578	Powder: tricalcium silicate, dicalcium silicate, tricalcium aluminate and calcium oxide Liquid: distilled water or liquid with additives (water and polyvinylpyrrolidone)	1g powder : 530 µL distilled water 1g powder : 530 µL liquid with additives
CL 2 to 30 µm + $CaWO_4$ + DW or LA	Clinker 2 to 30 µm + 30%$CaWO_4$ Angelus, Londrina, PR, Brazil Lot: 61186	Powder: tricalcium silicate, dicalcium silicate, tricalcium aluminate, calcium oxide and calcium tungstate Liquid: distilled water or liquid with additives (water and polyvinylpyrrolidone)	1g powder : 230 µL distilled water 1g powder : 230 µL liquid with additives
CL < 2 µm + $CaWO_4$ + DW or LA	Clinker < 2 µm + 30%$CaWO_4$ Angelus, Londrina, PR, Brazil Lot: 60578	Powder: tricalcium silicate, dicalcium silicate, tricalcium aluminate, calcium oxide and calcium tungstate Liquid: distilled water or liquid with additives (water and polyvinylpyrrolidone)	1g powder : 390 µL distilled water 1g powder : 390 µL liquid with additives
MTA	White MTA Angelus, Londrina, PR, Brazil	Powder: tricalcium silicate, dicalcium silicate, tricalcium aluminate, calcium oxide and calcium tungstate Liquid: distilled water	1g powder: 330 µL distilled water
MTAHP	MTA Repair HP Angelus, Londrina, PR, Brazil	Powder: tricalcium silicate, dicalcium silicate, tricalcium aluminate, calcium oxide and calcium tungstate Liquid with additives: water and polyvinylpyrrolidone	1g powder : 300 µL liquid with additives

CL: Clinker, $CaWO_4$: Calcium tungstate, DW: Distilled water, LA: Liquid with additives, MTAHP: MTA Repair HP

Evaluation of Physical Properties

Sample Size Calculation

The G*Power 3.1.7 program for Windows (Heinrich-Heine- Universität Dusseldorf, Dusseldorf, Germany) was used for sample calculation. One-way ANOVA was used with an alpha-type error of 0.05, and a beta power of 0.95 for all variables. The specific effect size for each variable was calculated: 2.83 for setting time, 2.11 for radiopacity, 0.96 for pH, and 1.68 for solubility (15). Six specimens per group were indicated as the ideal size required.

Setting Time

Setting time was evaluated according to ISO 6876:2012 (16) and ASTM C266-03 (17). After manipulation, the materials were inserted into plaster models (Type IV; Durone IV Salmon; Dentsply, Petropolis, RJ, Brazil) with a 10 mm in internal diameter and 1 mm height (n = 6). A Gilmore needle of 100 ± 0.5 g and 2 ± 0.1 mm diameter was used to determine the initial setting time, as established by the ISO 6876 standard. Subsequently, a Gilmore needle of 456 ± 0.5 g and 1. 0 ± 0.1 mm diameter was used to determine the final setting time, as established by ASTM C266-03. The initial and final setting times were considered in minutes, from the manipulation of the materials until the moment when the respective needles could not produce marks on the surface of the cements.

Radiopacity

After manipulation, the materials were inserted into circular plastic molds with a 10 mm in internal diameter and a 1 mm height (n = 6) and stored in an oven for 24 hours. After this period, the disc of each material and an aluminum scale with thickness ranging from 2 mm to 16 mm, in 2 by 2 step wedges, were placed on an occlusal film (Insight – Kodak Comp., Rochester, NY). A radiographic image (GE 1000 X-ray machine - General Electric, Milwaukee, WI) was performed using the following parameters: 60 kV, 7 mA, 0.32 pulses per second, and a focus-film distance of 33 cm. The films were processed, digitized, and evaluated using the Image J software (National Institutes of Health, Bethesda, USA).

Solubility

Circular plastic molds were made with 1.5 mm in height and 7.75 mm in internal diameter (18) and filled with each material (*n* = 6), inserting a nylon thread included in the mixture of each cement. The set was stored in an oven at 37 °C for 24 hours. The specimens were removed from the molds and weighed on a precision balance (Adventurer AR2140; Ohaus Corporation, Parsippany, NJ, USA) to determine the initial mass. Subsequently, the samples were suspended using nylon threads inside plastic containers with lids containing 7.5 mL of distilled and deionized water or phosphate-buffered saline (PBS). The containers remained in an oven at 37 °C for 7 days. After this period, the specimens were removed from the solutions, rinsed, and placed in a desiccator with silica. The samples were weighed every 24 hours until the final mass stabilized (0.001g). The percentage of solubility was determined using the following formula: [Percentage of solubility = Initial mass – final mass /initial mass * 100] (2, 19).

pH

Polyethylene tubes with open extremities (10 mm high and 1.6 mm diameter) were filled with each material (*n* = 10). Each tube was immersed in 10 mL of distilled water in a flask with a lid and kept in an oven at 37 °C. After each experimental period, the tubes were placed in a new flask with 10 mL of distilled and deionized water. The experimental periods were 1, 7, 14, 21 and 28 days. The pH of the solutions was measured using a previously calibrated digital pH meter (Mettler Toledo, Columbus, Ohio, USA). Flasks containing only distilled and deionized water were used as a control group.

Statistical Analysis

All data were submitted to the Shapiro-Wilk test and showed normal distribution. The One-way ANOVA and Tukey tests were used for comparisons between groups. To compare the different pH periods, repeated measures ANOVA and Tukey were used. The unpaired *t*- test was used for comparisons between distilled water or liquid with additives and comparisons between immersion solutions. The level of significance was 5% for all analyses.

RESULTS

Clinker 2 to 30 µm showed shorter initial setting time and longer final setting time when manipulated with liquid with additives ($P < .05$) (Tables 2). Clinker groups manipulated with distilled water showed shorter initial setting time compared to liquid with additives ($P < .05$) (Table 2). MTA showed a longer initial setting time than MTA Repair HP ($P < .05$).

The addition of calcium tungstate influenced the Clinker radiopacity for both particles ($P < .05$), but did not influence the setting time, solubility, and pH ($P > .05$) (Table 2). All materials showed mass gain ($P > .05$), except for MTA immersed in distilled water ($P < .05$). Clinker 2 to 30 µm + calcium tungstate showed greater mass gain and higher radiopacity when manipulated with liquid with additives than distilled water ($P < .05$). Distilled water provided less mass gain compared to PBS as an immersion solution ($P < .05$) (Table 2).

All materials presented alkaline pH ($P > .05$). Clinker < 2 µm promoted higher pH than Clinker 2 to 30 µm in some periods and groups ($P < .05$) (Table 3).

Table 2 - Mean and ± standard deviation of the initial and final setting time, radiopacity and solubility of different calcium silicate repair materials

	CL 2 to 30 µm	CL < 2 µm	CL 2 to 30 µm + 30 % $CaWO_4$	CL < 2 µm + 30 % $CaWO_4$	MTA / MTA HP
Initial setting time (min.)					
DW	15.0 ± 0.4^{bB}	11.6 ± 2.5^{bB}	18.3 ± 2.5^{bB}	12.5 ± 2.7^{bB}	41.5 ± 11.5^{aA}
LA	19.5 ± 2.5^{dA}	30.5 ± 3.3^{abA}	24.5 0.54^{cdA}	33.3 ± 4.63^{aA}	26.1 ± 4.2^{bcB}
Final setting time (min.)					
DW	166.0 ± 18.5^{bB}	121.1 ± 0.4^{cA}	200.3 ± 13.4^{bA}	107.8 ± 0.4^{cB}	245.4 ± 49.3^{aA}
LA	211.3 ± 6.8^{aA}	115.6 ± 13.0^{bB}	181.3 ± 6.7^{aB}	126.6 ± 0.5^{bA}	190.5 ± 52.5^{aA}
Radiopacity (mmAl)					
DW	1.85 ± 0.02^{bA}	1.83 ± 0.04^{bA}	3.80 ± 0.39^{aB}	3.57 ± 0.22^{aA}	4.01 ± 0.60^{aA}
LA	1.88 ± 0.04^{bA}	1.80 ± 0.01^{bA}	4.42 ± 0.18^{aA}	3.99 ± 0.49^{aA}	3.97 ± 0.59^{aA}
Solubility (%)					
Immersion solution – DW					
DW	-3.50 ± 1.92^{bA*}	-4.10 ± 1.55^{bA*}	-2.36 ± 1.88^{bA*}	-3.62 ± 1.14^{bA*}	3.21 ± 1.97^{aA*}
LA	-6.45 ± 2.75^{aA}	-4.52 ± 2.39^{aA}	-4.11 ± 1.41^{aB*}	-1.98 ± 1.65^{aA*}	-4.54 ± 1.68^{aB}
Immersion solution – PBS					
DW	-10.4 ± 2.06^{abA*}	-7.53 ± 2.75^{abA*}	-6.77 ± 1.19^{bA} *	-11.47 ± 4.64^{aA*}	-9.94 ± 1.73^{abA*}
LA	-8.17 ± 2.91^{aA}	-6.91 ± 3.17^{aA}	-9.83 ± 1.86^{aB*}	-7.06 ± 2.08^{aA*}	-7.12 ± 2.97^{aA}

CL: Clinker, CaWO4: calcium tungstate, MTAHP: MTA Repair HP, DW: Distilled water, LA: Liquid with additives, PBS: phosphate buffer saline.

Negative values in the solubility test indicate mass gain and positive values indicate mass loss

Different superscript lowercase letters in the same line indicate a significant difference between groups ($P < .05$). Different superscript uppercase capital in the same column indicates a significant difference between the different liquids for manipulation ($P < .05$). * Indicates a significant difference between immersion solution for each handling liquid ($P < .05$).

Table 3 - Mean and ± standard deviation of pH values obtained of the calcium silicate repair materials in different experimental periods

	Groups	DW	DW + 30 % $CaWO_4$	LA	LA + 30 % $CaWO_4$	#MTA HP +MTA	Control
24h	**CL 2 to 30 µm**	10.07 ± 0.35^{abA}	$10.16 \pm 0.18^{abA*}$	10.30 ± 0.12^{aA}	$10.04 \pm 0.32^{abA*}$	$^{\#}9.78 \pm 0.81^{bA}$	7.17 ± 0.18^{cA}
	CL < 2 µm	10.44 ± 0.24^{aA}	$10.41 \pm 0.25^{aA*}$	10.18 ± 0.52^{abA}	$10.56 \pm 0.22^{aA*}$	$^{+}10.05 \pm 0.2^{bA}$	7.16 ± 0.13^{cA}
7d	**CL 2 to 30 µm**	8.85 ± 0.44^{aB}	9.68 ± 0.35^{aB}	$9.74 \pm 0.41^{aB*}$	9.83 ± 0.50^{aA}	$^{\#}8.86 \pm 0.83^{bB*}$	$6.68 \pm 0.29^{cB*}$
	CL < 2 µm	9.38 ± 0.69^{aB}	9.57 ± 0.44^{aB}	$10.17 \pm 0.06^{aA*}$	9.52 ± 0.47^{aB}	$^{+}9.89 \pm 0.58^{aA*}$	$7.16 \pm 0.08^{bA*}$
14d	**CL 2 to 30 µm**	$8.29 \pm 0.24^{aB*}$	$8.60 \pm 0.28^{aC*}$	$8.46 \pm 0.27^{aC*}$	$8.58 \pm 0.41^{aB*}$	$^{\#}8.50 \pm 0.53^{aB*}$	$6.77 \pm 0.11^{bB*}$
	CL < 2 µm	$8.87 \pm 0.52^{bBC*}$	$9.05 \pm 0.46^{bC*}$	$9.78 \pm 0.09^{aA*}$	$9.40 \pm 0.38^{abB*}$	$^{+}8.98 \pm 0.26^{bB*}$	$7.02 \pm 0.18^{cA*}$
21d	**CL 2 to 30 µm**	$7.91 \pm 0.29^{bC*}$	$7.92 \pm 0.13^{bD*}$	$7.94 \pm 0.13^{bD*}$	$8.27 \pm 0.39^{abB*}$	$^{\#}8.34 \pm 0.49^{aB}$	$6.76 \pm 0.07^{cB*}$
	CL < 2 µm	$8.76 \pm 0.40^{bcBC*}$	$8.94 \pm 0.28^{bD*}$	$9.04 \pm 0.25^{abB*}$	$9.31 \pm 0.26^{aB*}$	$^{+}8.41 \pm 0.28^{cC}$	$6.23 \pm 0.13^{dB*}$
28d	**CL 2 to 30 µm**	$8.40 \pm 0.13^{abBC*}$	8.09 ± 0.03^{bD}	$8.12 \pm 0.17^{bD*}$	$8.62 \pm 0.36^{a\ B}$	$^{\#}8.34 \pm 0.46^{abB*}$	6.65 ± 0.27^{cB}
	CL < 2 µm	$8.24 \pm 0.30^{bC*}$	8.31 ± 0.41^{bD}	$8.62 \pm 0.32^{abB*}$	8.36 ± 0.58^{bC}	$^{+}9.05 \pm 0.34^{aB*}$	6.43 ± 0.46^{cB}

CL: Clinker, CaWO4: calcium tungstate, MTAHP: MTA Repair HP, DW: Distilled water, LA: Liquid with additives, PBS: phosphate buffer saline, h: hours, d: days.

Different superscript lowercase letters in the same line indicate a significant difference between groups ($P < .05$). Different superscript uppercase capital in the same column indicates a significant difference between the different experimental periods ($P < .05$). * indicates a statistical difference in the particle size of Clinker ($P < .05$).

DISCUSSION

The present study investigated the physical properties of Clinker Angelus in comparison with White MTA and MTA Repair HP. Particle size, the addition of a radiopacifier agent, as well as the influence of the liquid used for manipulation were explored to establish its clinical performance. Considering the different results between the materials evaluated regarding their physical properties, our null hypothesis was rejected.

The liquid with additives provided a shorter initial setting time for Clinker 2 to 30 µm, which can be related to changes in the hydration process of the material by the presence of the plasticizer in the water (2), accelerating the setting (20). However, the final setting time for Clinker 2 to 30 µm was longer when compared to Clinker <2 µm. This finding may be related to particle size, since larger particles have a smaller surface area, providing prolonged setting time (6). Another factor related to setting time is the powder/liquid ratio used to handle repair materials (12). In the present study, all materials manipulated with distilled water, except White MTA, showed shorter initial setting times when compared to liquid with additives. Conversely, a previous study

reported a longer setting time when materials were handled with distilled water (2). These differences may be related to material handling (12), and particle size with a direct impact on the setting reaction of calcium silicate-based materials (5, 8, 9). Achieving a balance in the setting time is important since a very short setting time affects the working time (21), while a longer setting time may increase the solubilization and dissolution of endodontic materials (8, 13).

Adequate radiopacity allows the material to be distinguished from adjacent anatomical structures (22). Our results showed radiopacity in accordance with ISO standards for all groups, except for Clinker without the association of calcium tungstate. Similar results were observed for Clinker, MTA, and MTA Repair HP (2, 19, 23). Different radiopacifier concentrations modify hydraulic materials' properties (3). The addition of 30% calcium tungstate to tricalcium silicate provides satisfactory physical and biological properties (2, 3). Therefore, in this study, 30% calcium tungstate was added to the Clinker, providing adequate radiopacity without changes in the other physical properties evaluated. The current results indicate that the liquid with additives positively influences the radiopacity of Clinker 2 to 30 µm. We can suggest that the polyvinylpyrrolidone used with the plasticizer of the MTA Repair HP liquid may have accelerated the hydration of the material and reduced the dissociation of the calcium tungstate. Furthermore, organic agents optimize the physical properties of calcium silicate-based materials (21, 24).

In the present study, all materials showed alkalinization capacity when compared to the control group, especially during the periods of 24 hours and 7 days. The alkalinity of Clinker may contribute to its biocompatibility and antimicrobial activity (25). Furthermore, a pH of 7.8 to 8.0 is considered ideal to obtain cell migration and consequently favors apical repair (26). Moreover, in this study, it was pointed out that Clinker <2 µm promoted a higher pH compared to Clinker 2 to 30 µm. Disagreeing with our results, a previous study reported that the addition of smaller particles decreases the release of hydroxyl ions (9). This result may be related to the sinter process of calcium silicate powder and the powder/liquid ratio used in the present study.

High solubility may enable bacterial recontamination of the root canal (12). Our results showed mass gain for all materials, except for White MTA. A balance between the release of calcium ions and solubility must be established to ensure the integrity of the material (12). Furthermore, calcium silicate-based cements are considered

hydrophilic, promoting continuous water absorption (12, 19). Although manipulation using liquid with additives optimizes the physical properties of materials, solubility is impaired (13). Higher solubility was reported for MTAHP (8.18%) when compared to MTA (4.91%) (13). Our results showed that manipulation with liquid with additives favored the solubility of Clinker 2 to 30 µm associated with calcium tungstate, in accordance with a previous study (19). In relation to the methodology recommended by ISO standards, distilled water is used to evaluate the mass loss of a material after a period of 24 hours (23, 27). Simulated body fluids are used to reproduce a physiological condition close to clinical reality (27, 28). Our results showed a greater mass gain in PBS than in distilled water. This result can be attributed to the water sorption potential of the material and the interaction of calcium ions from Bioceramic Clinker with phosphate from PBS (28, 29), promoting the formation of a surface layer of hydroxyapatite, which could lead to an increase in the material's mass.

This laboratory investigation revealed that particle size interferes with the physical behavior of materials, especially when manipulated with liquids with additives optimizing initial setting time, radiopacity, and solubility. Furthermore, our results suggest that Clinker has physical properties suitable for clinical applicability. Analyses focused on investigating the biological properties are important, therefore *in-vivo* studies should be developed to better understand the behavior of Clinker Angelus.

CONCLUSIONS

Clinker <2 µm manipulated with liquid with additives provides a shorter final setting time. Both particle sizes influence the alkalization capacity of Clinker, especially in the initial periods. The addition of calcium tungstate and manipulation with liquid with additives showed adequate physicochemical properties for Clinker.

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3.2 Publicação 2*

Physical changes of the Bioceramic Clinker Angelus after acid challenge using a dentin tube model. micro-CT and SEM analyzes

Abstract

This study evaluated the effect of immersion in butyric acid (BA, pH 4.1) or PBS (pH 7.0) on the properties of Clinker (CL) with particle sizes of 2 to 30 μm or < 2 μm associated with zirconium oxide and manipulated with distilled water (DW) or liquid with additives (LA) compared to Bio-C Repair (BCR) and Biodentine (BIO). Dentin tubes were filled with each material. After 24 hours, the specimens were immersed in BA or PBS (n = 5) for 7 and 28 days. Volumetric change, porosity, and material/dentin interface were evaluated by micro-CT, besides the analysis of the material surface by SEM. Kruskal-Wallis e Dunn, Mann-Whitney, Wilcoxon, unpaired *t*-test, and ANOVA e Tukey tests were performed (α=0,05). All groups exhibited volumetric changes similar to BCR and BIO (P > .05). BA showed greater increase in porosity (approximately 8%) than PBS (approximately 2%), except for CL2 to 30 μm with LA (P < .05). Immersion in BA for 28 days increased the porosity and the presence of gaps in the interface for all groups when compared to baseline (P < .05). CL 2 to 30 μm with DW showed greater porosity and gaps in the interface compared to the other groups (P < .05). SEM analysis revealed that all groups showed hydroxyapatite formation on the material surface in PBS and structural loss in BA. Acidic pH damages the material/dentin interface, porosity and promotes dimensional changes for bioceramics. Distilled water without additives increases porosity, interface gaps, and volume loss of Clinker.

KEYWORDS dental materials, endodontics, physical properties, x-ray microtomography

* Artigo escrito nas normas do periódico *Microscopy Research and Technique*, ao qual foi submetido.

1 INTRODUCTION

Calcium silicate-based materials are widely used due to their excellent biological and physicochemical properties (Donnermeyer, Bürklein, Dammaschke & Schäfer, 2019). Their adequate filling capacity and dimensional stability reduce the possibility of bacterial infiltration (Torres et al., 2020b; Torres, Pinto, Figueira, Guerreiro-Tanomaru & Tanomaru-Filho, 2021). Furthermore, the formation of an interfacial layer of hydroxyapatite resulting from the interaction of hydraulic cements with dentin improves the filling ability and highlights the bioactive potential of these materials (Reyes-Carmona, Felippe & Felippe, 2009).

Different manufacturers produce materials based on calcium silicates. Angelus bioceramic materials are produced from a powder called Clinker (CL; Angelus® Indústria de Produtos Odontológicos S/A, Londrina, PR, Brazil) with different particle sizes (2 to 30 µm or < 2 µm). Following the manufacturer, the raw materials go through homogenization, drying, and calcination processes until obtaining the clinker granule, composed of tricalcium silicate, dicalcium silicate, tricalcium aluminate, and calcium oxide. Then, this material is submitted to a grinding process to define the particle size (2 to 30 µm or < 2 µm) and associated with a radiopacifier agent. Adequate radiopacity, cytocompatibility, and bioactivity were reported for Clinker 2 to 30 µm associated with calcium tungstate as a radiopacifier after manipulation with distilled water or liquid with additives (Tanomaru-Filho et al., 2023). However, there are still no studies that evaluate the volumetric change, porosity, and material/dentin interface of Clinker Angelus.

The particle size and type of liquid used influence the properties of calcium silicate-based materials (Komabayashi & Spångberg, 2008; Ha, Kahler & Walsh, 2014; Ha, Bentz, Kahler & Walsh, 2015; Guimarães, Prati, Duarte, Bramante & Gandolfi, 2018; Inada et al., 2023). The presence of smaller particles in hydraulic repair cements improves the sealing capacity, reduces setting time, and film thickness, in addition to allowing greater flow and optimizing the antimicrobial potential (Komabayashi & Spångberg, 2008; Ha et al., 2015). In contrast, increasing particle size has been proven to adversely affect the setting time, pH, and compressive strength of Mineral trioxide aggregate (MTA) (Ha et al., 2014). Regarding the components employed to handle the material, the liquid with additives composed of water and a plasticizer improves the handling and insertion of the cement (Guimarães et al., 2018). However, greater solubility,

porosity, and gaps in the material/dentin interface have been reported when using liquid with additives (Guimarães et al., 2018; Inada et al., 2023). Until now, there is a lack in the literature evaluating the impact of particle size and type of liquid on the properties of Clinker, factors that are crucial for establishing adequate clinical applications.

Apical periodontitis is related to the maintenance of infection (Siqueira Jr & Rôças, 2022; dos Santos et al., 2024). Inflammation provides an acidic pH to periradicular tissues (Akinci, Simsek & Aydinbelge, 2020) and infection inside of dentinal tubules (Perez, Calas & Rochd, 1996; Siqueira Jr & Rôças, 2022), affecting the physicochemical properties of calcium silicate-based materials (Namazikhah et al., 2008; Ashofteh et al., 2019; Akinci et al., 2020, Silva, Pinto, Guerreiro-Tanomaru & Tanomaru-Filho, 2024). Modifications in crystal structures have been reported for ProRoot MTA (Dentsply Maillefer, Ballaigues, Switzerland), Biodentine (Septodont, Saint-Maur-des-Fossés, France), CEM Cement (Bionique, Tehran, Iran) and Retro MTA (BioMTA, Seoul, Republic of Korea) after exposure to acidic pH (Ashofteh et al., 2019). Furthermore, ProRoot MTA White (Dentsply Tulsa, Johnson City, TN, USA) showed higher porosity and low microhardness values after an acid challenge (Namazikhah et al., 2008). Moreover, Biodentine showed greater volume loss than ProRoot MTA White (Dentsply), BioAggregate (Innovative Bioceramix, Vancouver, Canada), and MTA (Angelus) after immersion in butyric acid (Akinci et al., 2020). However, to the best of our knowledge, the effect of acidic pH on the physical properties of Clinker Angelus remains unknown in the literature.

Considering that repair cements based on calcium silicate can come into contact with an acidic environment in the presence of periradicular inflammation, the aim of this study was to evaluate the effect of acidic pH on the volumetric change, porosity, and material/dentin interface of Clinker with particle size from 2 to 30 μm or < 2 μm associated with the zirconium oxide radiopacifier and manipulated with distilled water or liquid with additives using micro-computed tomography (micro-CT). Bio-C Repair and Biodentine were used as references for comparisons. Analysis of the material surface was evaluated using scanning electron microscopy (SEM). The null hypotheses were: 1) the immersion solution would not influence the physical properties of the materials; 2) There would be no difference between the groups in relation to properties; and 3) the immersion time would not influence the variables evaluated.

2 MATERIAL AND METHODS

The experimental groups, materials with their respective manufacturers, compositions, proportions, and immersion solutions are described in Table 1

Table 1 - Experimental groups, material with their respective manufacturers, composition, proportion, and immersion solution

Groups	Material and Manufacturers	Composition	Proportion	Immersion Solution
CL 2 to 30 µm + 30% ZrO_2 + DW	Clinker 2 to 30 µm Angelus, Londrina, PR, Brazil Lot: 62965	Powder: tricalcium silicate, dicalcium silicate, tricalcium aluminate, calcium oxide and zirconium oxide Liquid: distilled water	1g : 450 µL (powder/distilled water)	Butyric acid PBS
CL 2 to 30 µm + 30% ZrO_2 + LA	Clinker 2 to 30 µm Angelus, Londrina, PR, Brazil Lot: 62965	Powder: tricalcium silicate, dicalcium silicate, tricalcium aluminate, calcium oxide and zirconium oxide Liquid: water and plasticizer (polyvinylpyrrolidone)	1g : 450 µL (powder/liquid with additives)	Butyric acid PBS
CL < 2 µm + 30% ZrO_2 + DW	Clinker < 2 µm Angelus, Londrina, PR, Brazil Lot: 64633	Powder: tricalcium silicate, dicalcium silicate, tricalcium aluminate, calcium oxide and zirconium oxide Liquid: distilled water	1g : 450 µL (powder/distilled water)	Butyric acid PBS
CL < 2 µm + 30% ZrO_2 + LA	Clinker < 2 µm Angelus, Londrina, PR, Brazil Lot: 64633	Powder: tricalcium silicate, dicalcium silicate, tricalcium aluminate, calcium oxide and zirconium oxide Liquid: water and plasticizer (polyvinylpyrrolidone)	1g : 450 µL (powder/liquid with additives)	Butyric acid PBS
BCR	Bio-C Repair Angelus, Londrina, PR, Brazil Lot: 57779 and 56446	Calcium silicates, calcium aluminate, calcium oxide, zirconium oxide, iron oxide, silicon dioxide, and dispersing agent	Ready to use	Butyric acid PBS
BIO	Biodentine Septodont, Saint Maur des Fossés, France Lot: B2622OA	Powder: tricalcium silicate, calcium oxide, calcium carbonate, iron oxides pigment and zirconium oxide Liquid: calcium chloride and polycarboxylate	1 capsule : 6 drops of liquid	Butyric acid PBS

CL: Clinker, ZrO_2: zirconium oxide, DW: distilled water, LA: liquid with additives, BCR: Bio-C Repair, BIO: Biodentine, PBS: phosphate buffer solution

2.1 Sample size calculation

The G*Power 3.1.7 program for Windows (Heinrich-Heine-Universitat Dusseldorf, Dusseldorf, Germany) was used for the sample calculation. One-way ANOVA test was used with an Alpha-type error of 0.05 and Beta power of 0.95 for all variables. Previous studies were used to determine the specific effect size has on volumetric change, 0.98 (Torres et al., 2020b); porosity, 2.21; and gaps in the interface, 1.20 (Inada et al., 2023). A total of 5 specimens per group for all variables analyzed was indicated as the ideal size required to observe significant differences .

2.2 Selection and preparation of specimens

After approval by the Ethics Committee for the Use of Animals (n° 28/2020), sixty extracted bovine teeth were radiographed (Kodak RVG 6100 Digital Radiography System, Marne-la-Vallée, France) to confirm the absence of anomalies. The middle third of each root was transversely sectioned using an Isomet 1000 cutting machine (Buehler Ltd, Lake Bluff, IL, USA). A single previously calibrated operator prepared the root canals with a Gates-Glidden drill number 6 (Dentsply Maillefer, Ballaigues, Switzerland) coupled to the motor and low rotation (Micromotor N270 and Contra-angle; Dabi-Atlante, Ribeirão Preto, São Paulo, Brazil) and with the aid of the delineator device (Bio-Art, São Carlos, São Paulo, Brazil) to ensure the fixation of the samples (Vivan et al., 2016). All walls of the dentin tubes were standardized to approximately 1 mm in thickness using a cylindrical drill (Maxicut 1503; American Burrs, Palhoça, Santa Catarina, Brazil) and confirmed by a digital caliper (Mitutoyo Corporation; São Paulo, SP, Brazil) and digital radiography (Kodak RVG 6100 Digital Radiography System). All specimens were 4 mm in length and 1.5 mm in internal diameter, as illustrated in Figure 1(A). During the entire preparation, the canals were irrigated with a 1% sodium hypochlorite (NaOCl) solution using an Ultradent syringe (South Jordan, UT, USA) with a 30G Navitip needle (Ultradent). The final irrigation was performed with 5 mL of 2.5% NaOCl and 5 mL of 17% EDTA for 3 minutes, followed by 5 mL of distilled water. Subsequently, the samples were immersed in distilled water and stored in an oven at 37°C for 24 hours (Silva et al., 2024).

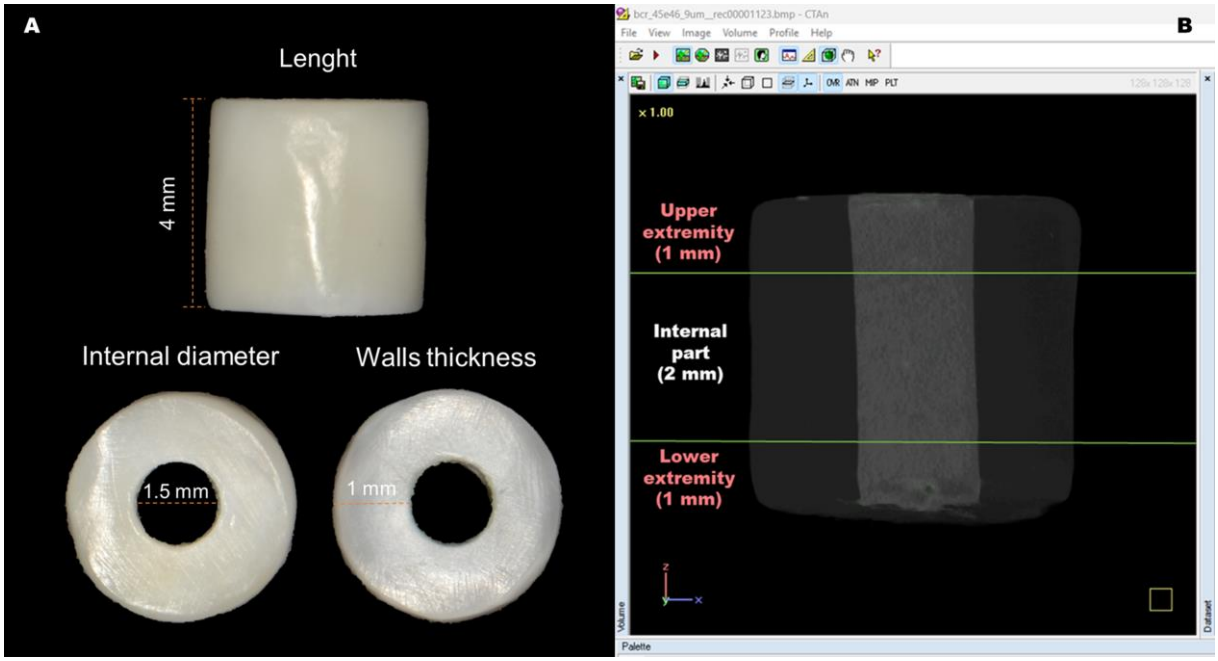

Figure 1 - Representative image of dentin tubes. (A) parameters of bovine dentin tubes and (B) division of the sample for analysis of variables using the CTAn software

2.3 Filling of the specimens

Dentin tubes were filled with CL 2 to 30 µm + ZrO_2 + DW; CL 2 to 30 µm + ZrO_2 + LA; CL < 2 µm + ZrO_2 + DW; CL < 2 µm + ZrO*2* + LA; BCR or BIO (*n* = 10) using a condenser kit (Ref.: 324501, nº 2, 3 and 4; Golgran; São Caetano do Sul, SP, Brazil) and stored in an oven at 37°C for 24 hours (Silva et al., 2024).

2.4 Micro-CT scan and immersion of the specimens

The specimens were submitted for initial micro-CT scanning (SkyScan 1272, Bruker, Kontich, Belgium) with voxel size 8.74 µm, rotation step 0.5, frame 3, Al filter 1 mm, rotation 180°, 80 kV and 125 uA. Then, each specimen was inserted individually into the Eppendorf tube with 1.5 mL of butyric acid (Sigma Aldrich, Barueri, SP, Brazil, pH: 4.1) or PBS (Sigma, pH: 7.0) (*n* = 5) and stored in an oven at 37 ºC for 7 and 28 days. The butyric acid solution was changed every 24 hours throughout the experiment to ensure pH stability (Silva et al., 2024). Micro-CT scans after 7 and 28 days of sample immersion were performed using the same parameters described previously.

2.5 Micro-CT analysis

Image reconstructions in the different experimental periods were performed using the NRecon v.1.6.3 software (Bruker micro-CT), after defining individual parameters for each material. The 3D reconstructed images were recorded at different periods using the Data Viewer v.1.5.1 software (Bruker micro-CT). Quantitative analyses before and after immersion in different solutions were carried out using the software CTAn v.1.15.4.0 (Bruker micro-CT). Representative images were created employing CTVox software v.3.2 (Bruker micro-CT). All dentin tubes were divided into three parts: 1 mm for the upper extremity, 1 mm for the lower extremity (extremities), and 2 mm for the center of the sample (internal part), as illustrated in Figure 1(A).

The volumetric change and porosity were calculated by determining the initial volume (mm^3) and, after immersion for 7 and 28 days in the different solutions, obtaining values in mm^3 and percentage. The percentage of gaps in the material/dentin interface was determined based on the methodology described by previous study (Gandolfi, Parrilli, Fini, Prati & Dummer, 2013). The 3D distribution of the interface of gaps in a predefined volume of interest (VOI) was calculated after filling and after immersion in butyric acid or PBS for 7 and 28 days. Each 3D-VOI was defined as the interface volume, encompassing part of the dentin and part of the filling material through a specific "*task list*" with arithmetic and logical operations between the superimposed sections. In this way, gaps with sizes starting at 8.74 µm inside the VOI were detected. For all analyses, the grayscale required to recognize each object of interest was determined using a density histogram employing an adaptive threshold.

2.6 Surface analysis by SEM

After immersion for 28 days in butyric acid or PBS (n = 3 from each group), the samples were dried with absorbent paper and kept in a desiccator (Plena-Lab, São Paulo, SP, Brazil) containing silica for 30 days. The specimens were coated with carbon and viewed in SEM (JEOL, JSM – 6610LV Scanning Electron Microscope, Peabody, MA, USA) at 12 kV and SS 40. The surface of each sample was examined using three magnifications (x27, x100, and x500).

2.7 Data analysis

All data were submitted to the Shapiro-Wilk test. Data relative to the percentage of gaps in the material/dentin interface and porosity at the extremities of the samples after 28 days showed a normal distribution. One-way ANOVA and Tukey were used for comparisons between groups, and the unpaired *t*-test was used for comparisons between immersion solutions. The data relative to the percentage of volumetric change, porosity after immersion for 7 days, porosity of the internal part of the samples after 28 days, and percentage of porosity increase after immersion in butyric acid or PBS presented a non-normal distribution. The Kruskal-Wallis and Dunn tests were used to compare groups, and the Mann-Whitney test was used to compare immersion solutions. Finally, the Wilcoxon test was used to compare materials before and after the immersion periods. The significance level was 5% for all analyses.

3 RESULTS

3.1 Volumetric change

All cements showed similar volumetric changes to BCR and BIO regardless of the immersion solutions and evaluation period ($P > .05$). CL<2+ZrO_2+DW showed greater volumetric loss in periods of 7 and 28 days compared to CL2 to 30+ZrO_2+LA at the extremities of the samples ($P < .05$). Immersion in butyric acid showed volumetric loss for BCR and CL< 2 manipulated with DW or LA after 7 days ($P < .05$) and also for all groups after 28 days when compared with PBS at the extremities of the samples ($P < .05$). (Figure 2, Tables 2 and 3).

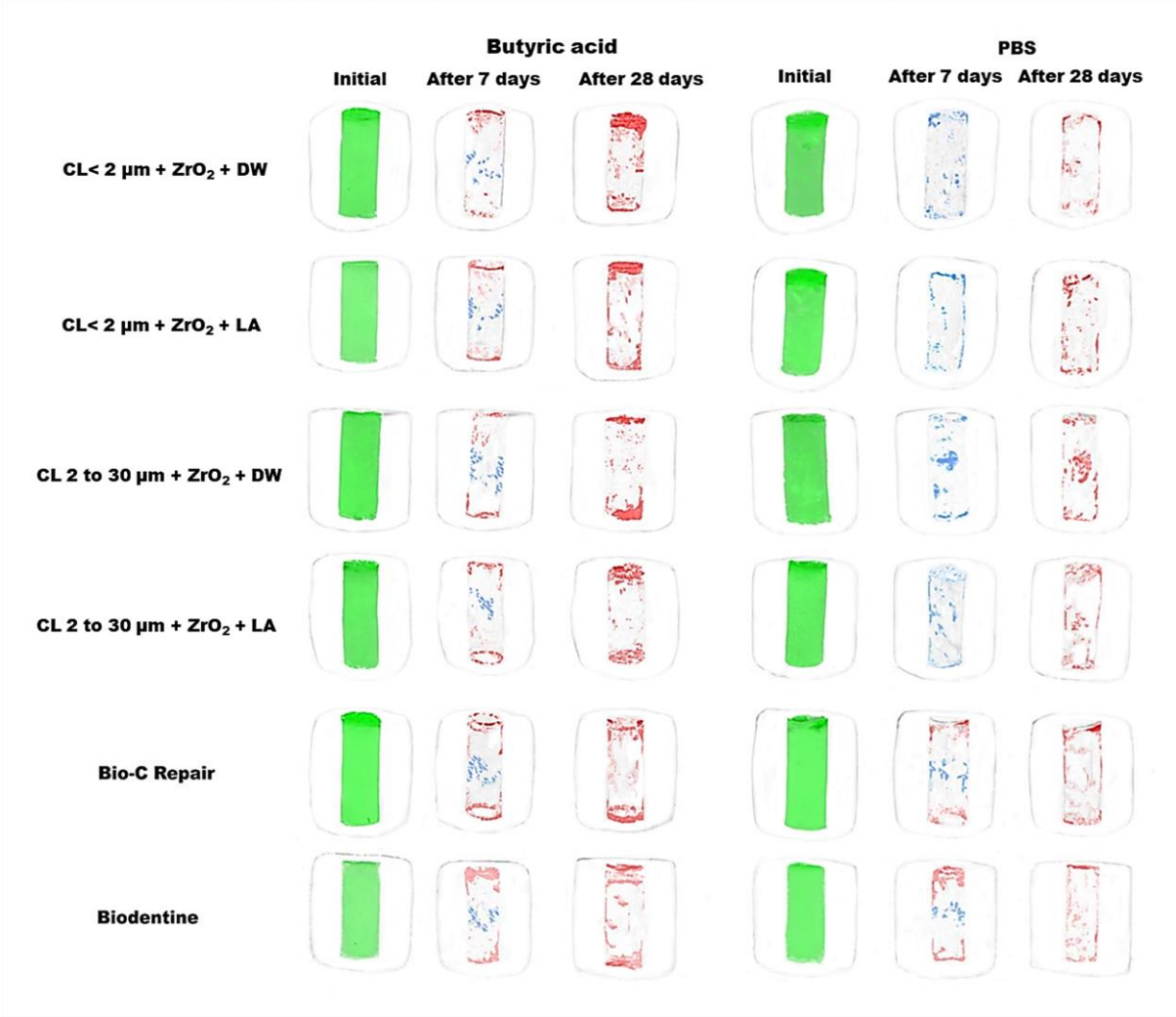

Figure 2 - 3D models showing the initial volume (green), volume gain (blue) and volumetric loss (red) of the materials evaluated before and after immersion in butyric acid or PBS for periods of 7 and 28 days

Table 2 - Median, minimum and maximum values of the percentage of volumetric change of materials evaluated after immersion in butyric acid (BA) or PBS for 7 days

	Extremities	
	BA	PBS
CL < 2 μm + ZrO_2 + DW	-4.13 (-14.8-(-0.02))^{bB}	1.67 (-1.59-3.00)^{aA}
CL < 2 μm + ZrO_2+ LA	-2.53 (-5.76-(-1.06))^{abB}	0.14 (-1.44-2.55)^{abA}
CL 2 to 30 μm + ZrO_2+ DW	-1.97 (-6.80-1.07)^{abA}	0.28 (-5.42-4.78)^{abA}
CL 2 to 30 μm + ZrO_2+ LA	-1.35 (-3.77-0.41)^{aA}	0.69 (-3.54-3.79)^{aA}
Bio-C Repair	-4.43 (-5.75-(-0.42))^{bB}	-0.10 (-2.28-1.21)^{abA}
Biodentine	-3.40 (-8.07-(-0.60))^{abA}	-1.19 (-5.89-0.67)^{bA}
	Internal part	
	BA	PBS
CL < 2 μm + ZrO_2 + DW	3.55 (2.86-3.72)^{aA}	0.42 (-1.38-1.21)^{bB}
CL < 2 μm + ZrO_2+ LA	1.87 (1.80-3.73)^{aA}	3.59 (1.78-3.77)^{aA}
CL 2 to 30 μm + ZrO_2+ DW	3.03 (1.76-3.72)^{aA}	3.55 (3.47-3.68)^{aA}
CL 2 to 30 μm + ZrO_2+ LA	1.86 (1.80-3.80)^{aA}	3.64 (1.81-3.68)^{aA}
Bio-C Repair	3.57 (1.70-3.63)^{aA}	3.68 (1.80-3.79)^{aA}
Biodentine	2.78 (1.80-3.44)^{aA}	1.82 (1.64-3.82)^{aA}

CL: Clinker, ZrO_2: zirconium oxide, DW: distilled water, LA: liquid with additives, PBS: phosphate buffer solution.
Negative values: volumetric loss, Positive values: volumetric gain
Different superscript lowercase letters in the same column indicate a significant difference between groups ($p < 0.05$). Different superscript uppercase letters in the same line indicate a significant difference between the immersion solutions ($p < 0.05$).

Table 3 - Median, minimum and maximum values of the percentage of volumetric change of materials evaluated after immersion in butyric acid (BA) or PBS for 28 days

	Extremities	
	BA	PBS
CL < 2 μm + ZrO_2 + DW	-20.20 (-41.34-(-8.67))^{aB}	-0.05 (-1.71-4.35)^{aA}
CL < 2 μm + ZrO_2+ LA	-15.18 (-5.76-(-1.06))^{abB}	-1.37 (-12.57-3.80)^{aA}
CL 2 to 30 μm + ZrO_2+ DW	-12.89 (-34.01-(-4.43))^{abB}	-1.01 (-12.51-3.27)^{aA}
CL 2 to 30 μm + ZrO_2+ LA	-12.74 (-19.13-(-5.05))^{bB}	-0.97 (-3.54-1.48)^{aA}
Bio-C Repair	-13.50 (-32.85-(-9.19))^{abB}	-1.06 (-6.97-3.74)^{aA}
Biodentine	-15.17 (-37.13-(-9.19))^{abB}	-0.13 (14.28-3.18)^{aA}
	Internal part	
	BA	PBS
CL < 2 μm + ZrO_2 + DW	-0.98 (-2.92-(-0.38))^{aA}	-0.78 (-3.29-(-0.31))^{aA}
CL < 2 μm + ZrO_2+ LA	-0.50 (-1.24-0.18)^{abA}	-1.01 (-1.92-0.80)^{aA}
CL 2 to 30 μm + ZrO_2+ DW	-0.44 (-3.67-3.31)^{abA}	-3.15 (-6.45-(-0.20))^{aA}
CL 2 to 30 μm + ZrO_2+ LA	-0.11 (-0.22-0.85)^{bA}	-1.73 (-1.91-(-0.41))^{aB}
Bio-C Repair	-0.40 (-0.75-0.47)^{abA}	-0.26 (-2.16-0.73)^{aA}
Biodentine	-0.36 (-1.09-1.14)^{abA}	-0.40 (-8.52-0.06)^{aA}

CL: Clinker, ZrO_2: zirconium oxide, DW: distilled water, LA: liquid with additives, PBS: phosphate buffer solution.
Negative values: volumetric loss, Positive values: volumetric gain
Different superscript lowercase letters in the same column indicate a significant difference between groups ($p < 0.05$). Different superscript uppercase letters in the same line indicate a significant difference between the immersion solutions ($p < 0.05$).

3.2 Porosity

CL2 to 30+ZrO_2+DW demonstrated greater porosity compared to the other groups in all evaluation periods ($P < .05$). All groups, except BCR after 28 days, showed an increase in porosity in both evaluation periods in relation to the baseline ($P < .05$). CL2 to 30+ZrO_2+DW and BIO showed greater porosity in butyric acid than PBS at the extremities ($P < .05$), and lower initial porosity in relation to the period of 28 days in the internal part of the samples ($P < .05$) (Figure 3, Tables 4 and 5).

When immersed in butyric acid, a porosity increases of approximately 8% was observed for all groups at the extremities of the samples, except for CL2 to

30+ZrO_2+LA. On the other hand, an increase of approximately 2% was observed in the porosity of the samples after immersion in PBS for 28 days ($P < .05$). There were no differences regarding in the internal part after immersion in both solutions ($P > .05$). These results are illustrated at Figure 4.

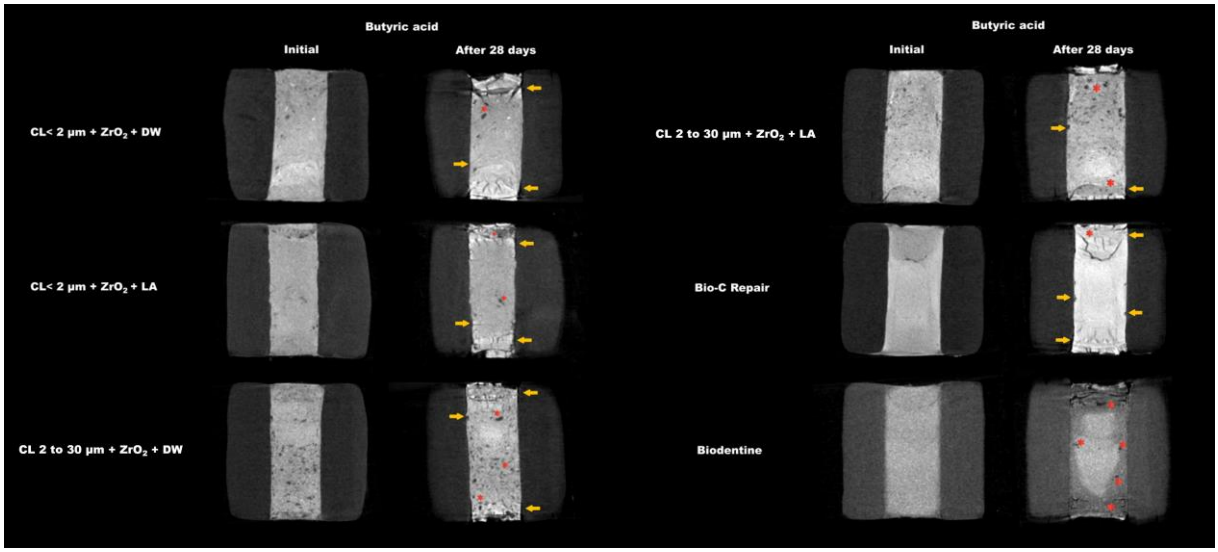

Figure 3 - Representative image of porosity (red asterisks) and gaps in the material/dentin interface (yellow arrows) of the different groups after immersion in butyric acid for a period of 28 days

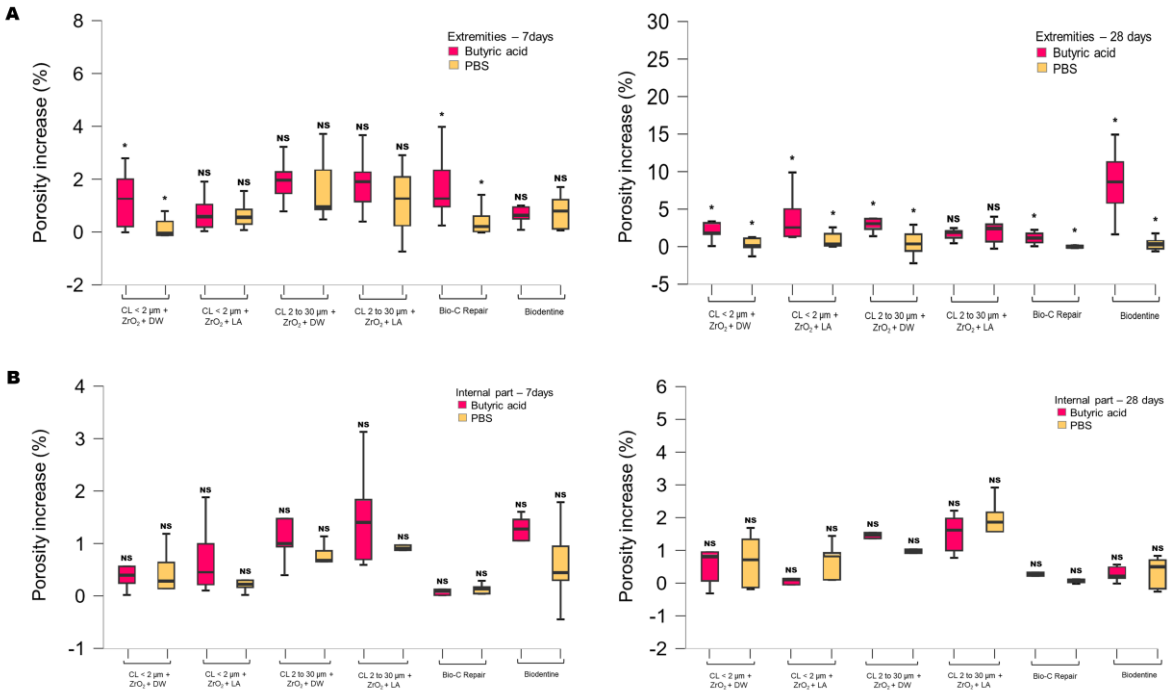

Figure 4 – Boxplots representing the median, minimum, and maximum of the porosity increase (%) at the extremities (A) and internal part (B) of the samples filled with repair materials after immersion in butyric acid or PBS for a period of 7 and 28 days. Asterisk (*) indicate statistical difference between immersion solutions ($P < .05$). NS indicate non-significance between butyric acid and PBS ($P > .05$).

Table 4 - Median, minimum and maximum values of the percentage of porosity observed in the extremities and internal part of the materials evaluated before and after immersion in butyric acid (BA) or PBS for 7 days

	Extremities			
	BA	PBS	BA	PBS
	Before immersion		**After immersion**	
CL < 2 µm + ZrO_2 + DW	0.86 (0.11-1.86)^{bB}	1.58 (0.21-3.79)^{bcB}	1.88 (0.22-8.66)^{bcA}	2.00 (0.19-3.86)^{bA}
CL < 2 µm + ZrO_2+ LA	0.68 (0.06-4.45)^{bB}	0.87 (1.15-3.00)^{bcB}	1.48 (1.28-6.36)^{bcA}	1.49 (0.28-4.56)^{bA}
CL 2 to 30 µm + ZrO_2+ DW	4.05 (2.51-11.16)^{aB}	5.30 (1.07-9.77)^{aB}	6.55 (4.20-13.13)^{aA}	7.02 (2.94-11.76)^{aA}
CL 2 to 30 µm + ZrO_2+ LA	2.02 (0.80-4.77)^{aB}	1.17 (1.38-4.92)^{bB}	4.52 (1.65-5.81)^{abA}	3.44 (1.51-7.83)^{aA}
Bio-C Repair	0.65 (0.01-2.15)^{bB}	0.85 (0.04-4.33)^{bcB}	2.35 (0.25-5.12)^{bA}	1.13 (0.07-5.02)^{bA}
Biodentine	0.59 (0.07-1.52)^{bB}	0.94 (0.31-3.87)^{cB}	1.23 (0.57-4.59)^{bcA}	1.83 (0.41-4.64)^{bA}
	Internal part			
	BA	PBS	BA	PBS
	Before immersion		**After immersion**	
CL < 2 µm + ZrO_2 + DW	0.37 (0.10-1.69)^{bc}	0.99 (0.32-1.68)^{bc}	0.62 (0.12-4.50)^{bc}	0.99 (0.60-2.42)^{bc}
CL < 2 µm + ZrO_2+ LA	0.06 (0.01-1.19)^{c}	0.13 (0.01-1.09)^{c}	0.27 (0.16-2.04)^{c}	0.25 (0.03-2.39)^{c}
CL 2 to 30 µm + ZrO_2+ DW	4.27 (2.67-4.79)^{a}	3.59 (2.40-6.92)^{a}	5.73 (3.07-7.76)^{a}	4.45 (3.08-7.09)^{a}
CL 2 to 30 µm + ZrO_2+ LA	1.93 (0.75-2.74)^{ab}	2.10 (1.43-3.50)^{ab}	2.63 (2.39-5.29)^{ab}	2.55 (2.25-6.44)^{ab}
Bio-C Repair	0.15 (0.01-1.56)^{c}	0.65 (0.01-0.91)^{c}	0.28 (0.01-2.09)^{c}	0.82 (0.04-1.20)^{c}
Biodentine	0.19 (0.09-1.49)^{bc}	0.67 (0.12-1.72)^{bc}	1.69 (0.46-2.94)^{bc}	1.29 (0.82-2.46)^{bc}

CL: Clinker, ZrO_2: zirconium oxide, DW: distilled water, LA: liquid with additives, PBS: phosphate buffer solution. Different superscript lowercase letters in the same column indicate a significant difference between groups ($p < 0.05$). Different superscript uppercase letters in the same line indicate a significant difference between before and after immersion in the extremities ($p < 0.05$). There was no significant difference between the immersion solutions in the extremities of the samples ($p > 0.05$). There was no significant difference between the immersion solutions and between before and after immersion in the internal part of the samples ($p > 0.05$).

Table 5 - Percentage of porosity of the materials evaluated before and after immersion in butyric acid (BA) or PBS for 28 days

	Extremities			
	BA	PBS	BA	PBS
	Before immersion		**After immersion**	
CL < 2 μm + ZrO_2 + DW	1.03 ± 0.56^{bA*}	1.39 ± 0.64^{bA}	2.40 ± 1.18^{cA*}	1.73 ± 0.86^{cA}
CL < 2 μm + ZrO_2+ LA	1.13± 0.82^{bA*}	1.60 ± 1.15 ^{bA}	4.28 ± 2.34^{bcA*}	2.52 ± 1.14^{bcA}
CL 2 to 30 μm + ZrO_2+ DW	5.92 ± 2.47^{aA*}	5.40 ± 2.05^{aA}	9.70 ± 4.20^{aA*}	6.16 ± 2.08^{aB}
CL 2 to 30 μm + ZrO_2+ LA	2.08 ± 1.28^{bA*}	3.10 ± 1.05^{bA*}	3.76 ± 1.17^{cA*}	5.05 ± 2.12^{aA*}
Bio-C Repair	1.02± 0.57^{bA}	1.56 ± 0.58^{bA}	1.80 ± 1.02^{cA}	1.62 ± 1.15^{cA}
Biodentine	0.41± 0.19^{bA*}	1.16 ± 0.92^{bA}	8.82 ± 3.58^{abA*}	1.28 ± 0.45^{cB}
	Internal part			
	BA	PBS	BA	PBS
	Before immersion		**After immersion**	
CL < 2 μm + ZrO_2 + DW	1.02 (0.28-1.50)^{bA}	0.77 (0.24-1.29)^{bA}	2.38 (0.35-6.21)^{abA}	0.94 (0.64-2.63)^{bcA}
CL < 2 μm + ZrO_2+ LA	0.16 (0.09-1.03)^{bcB}	1.67 (0.34-3.18)^{abA}	0.26 (0.11-0.71)^{cB}	2.57 (0.44-4.62)^{abA}
CL 2 to 30 μm + ZrO_2+ DW	4.22 (2.68-4.68)^{abA*}	2.90 (2.40-5.98)^{aA}	5.77 (4.10-6.58)^{aA*}	5.61 (1.02-6.54)^{aA}
CL 2 to 30 μm + ZrO_2+ LA	1.46 (0.65-2.25)^{bB}	3.13(2.26-4.08)^{aA*}	2.83 (1.76-4.46)^{abB}	4.99 (3.19-7.99)^{aA*}
Bio-C Repair	0.34 (0.01-1.16)^{bcA}	0.65 (0.07-0.91)^{bA}	1.39 (0.04-1.67)^{bcA}	0.63 (0.02-1.01)^{cA}
Biodentine	0.10 (0.04-0.34)^{cA*}	0.36 (0.10-1.74)^{bA}	0.51 (0.11-0.67)^{cA*}	0.80 (0.10-1.57)^{cA}

Mean and **±** standard deviation of the percentage of porosity in the extremities
Median, minimum, and maximum values of the percentage of porosity in the internal part
CL: Clinker, ZrO_2: zirconium oxide, DW: distilled water, LA: liquid with additives, PBS: phosphate buffer solution. Different superscript lowercase letters in the same column indicate a significant difference between groups ($p < 0.05$). Different superscript uppercase letters in the same line indicate a significant difference between the immersion solutions ($p < 0.05$). * indicates a difference between before and after immersion ($p < 0.05$).

3.3 Material/dentin interface

Baseline showed a lower percentage of gaps when compared to the experimental groups ($P < .05$). After 28 days, butyric acid was shown to negatively influence the quality of the material/dentin interface at the extremities of the samples ($P < .05$). CL2 to 30+ZrO_2+DW showed a higher percentage of gaps in the interface compared to the other groups at the extremities of the samples ($P < .05$). A higher percentage of interface gaps for BCR were observed in butyric acid compared to PBS in both evaluation periods ($P < .05$). (Figure 3, Tables 6 and 7).

Table 6 - Mean and ± standard deviation of the percentage of gaps in the material/dentin interface observed in the extremities and internal part of the materials evaluated before and after immersion in butyric acid (BA) or PBS for 7 days

	Extremities			
	BA	PBS	BA	PBS
	Before immersion		**After immersion**	
CL < 2 µm + ZrO_2 + DW	3.53 ± 0.78^{bA}	2.99 ± 0.94^{bcA}	6.03 ± 1.30^{bcB}	5.11 ± 1.52^{abB}
CL < 2 µm + ZrO_2+ LA	3.24 ± 1.55^{bA}	3.41 ± 0.63^{bcA}	5.76 ± 1.29^{cB}	4.77 ± 1.17^{bB}
CL 2 to 30 µm + ZrO_2+ DW	6.29 ± 1.91^{aA}	5.51 ± 1.09^{aA}	9.22 ± 1.53^{aB}	7.16 ± 1.81^{aB}
CL 2 to 30 µm + ZrO_2+ LA	4.84 ± 0.88^{abA}	4.69 ± 1.83^{abA}	8.11 ± 1.48^{abB}	6.11 ± 1.85^{abB}
Bio-C Repair	$3.84 \pm 0.83^{bA*}$	$2.89 \pm 0.89^{cA*}$	$5.80 \pm 1.18^{bcB*}$	$3.87 \pm 1.19^{bB*}$
	Internal part			
	BA	PBS	BA	PBS
	Before immersion		**After immersion**	
CL < 2 µm + ZrO_2 + DW	3.24 ± 0.57^{bB}	3.97 ± 1.30^{aB}	5.66 ± 1.35^{abA}	4.97 ± 2.39^{aA}
CL < 2 µm + ZrO_2+ LA	3.09 ± 1.70^{bB}	3.23 ± 0.99^{aB}	5.08 ± 1.94^{bA}	4.76 ± 1.39^{aA}
CL 2 to 30 µm + ZrO_2+ DW	6.36 ± 1.62^{aB}	5.29 ± 1.81^{aB}	8.47 ± 2.47^{aA}	7.02 ± 1.80^{aA}
CL 2 to 30 µm + ZrO_2+ LA	4.73 ± 0.76^{abB}	4.51 ± 2.12^{aB}	7.93 ± 1.46^{abA}	5.95 ± 2.63^{aA}
Bio-C Repair	3.84 ± 0.82^{bB}	2.70 ± 0.96^{aB}	$5.79 \pm 1.36^{abA*}$	$3.61 \pm 1.34^{aA*}$

CL: Clinker, ZrO_2: zirconium oxide, DW: distilled water, LA: liquid with additives, PBS: phosphate buffer solution. Different superscript lowercase letters in the same column indicate a significant difference between groups ($p < 0.05$). Different superscript uppercase letters in the same line indicate a significant difference between before and after immersion ($p < 0.05$). * Indicates a significant difference between immersion solutions ($p < 0.05$).

Table 7 - Mean and ± standard deviation of the percentage of gaps in the material/dentin interface observed in the extremities and internal part of the materials evaluated before and after immersion in butyric acid (BA) or PBS for 28 days

	Extremities			
	BA	PBS	BA	PBS
	Before immersion		**After immersion**	
CL < 2 µm + ZrO_2 + DW	3.53 ± 0.78^{bA}	2.99 ± 0.94^{bA}	13.89 ± 4.14^{abB*}	7.21 ± 2.82^{abB*}
CL < 2 µm + ZrO_2+ LA	3.24 ± 1.55^{bA}	3.41 ± 0.63^{bcA}	13.14 ± 3.47^{bB*}	7.29 ± 2.90^{abB*}
CL 2 to 30 µm + ZrO_2+ DW	6.29 ± 1.91^{aA}	5.51 ± 1.09^{aA}	17.59 ± 1.91^{aB*}	9.28 ± 1.72^{abB*}
CL 2 to 30 µm + ZrO_2+ LA	4.84 ± 0.88^{abA}	4.69 ± 1.83^{abA}	14.51 ± 2.71^{abB*}	9.80 ± 3.07^{abB*}
Bio-C Repair	3.84 ± 0.83^{bA*}	2.89 ± 0.89^{cA*}	12.53 ± 3.15^{bB*}	6.01 ± 1.74^{bB*}
	Internal part			
	BA	PBS	BA	PBS
	Before immersion		**After immersion**	
CL < 2 µm + ZrO_2 + DW	3.24 ± 0.57^{bB}	3.97 ± 1.30^{aB}	11.57 ± 2.12^{aA*}	7.20 ± 3.06^{aA*}
CL < 2 µm + ZrO_2+ LA	3.09 ± 1.70^{bB}	3.23 ± 0.99^{aB}	6.99 ± 1.95^{bA}	7.31 ± 3.40^{aA}
CL 2 to 30 µm + ZrO_2+ DW	6.36 ± 1.62^{aB}	5.29 ± 1.81^{aB}	10.15 ± 3.29^{abA}	9.99 ± 1.83^{aA}
CL 2 to 30 µm + ZrO_2+ LA	4.73 ± 0.76^{abB}	4.51 ± 2.12^{aB}	8.44 ± 1.94^{abA}	9.10 ± 3.31^{aA}
Bio-C Repair	3.84 ± 0.82^{bB}	2.70 ± 0.96^{aB}	8.43 ± 1.12^{abA*}	5.75 ± 1.55^{aA*}

CL: Clinker, ZrO_2: zirconium oxide, DW: distilled water, LA: liquid with additives, PBS: phosphate buffer solution. Different superscript lowercase letters in the same column indicate a significant difference between groups ($p < 0.05$). Different superscript uppercase letters in the same line indicate a significant difference between before and after immersion ($p < 0.05$). * Indicates significant difference between immersion solutions ($p < 0.05$).

3.4 Surface analysis by SEM

Descriptive analysis by SEM revealed the formation of hydroxyapatite crystals on the surface of the samples when immersed in PBS, in addition to a continuous interfacial layer between the material and dentin. On the other hand, structural loss, corrosion of the material, and the formation of cracks at the material/dentin interface were observed in all groups immersed in butyric acid (Figure 5).

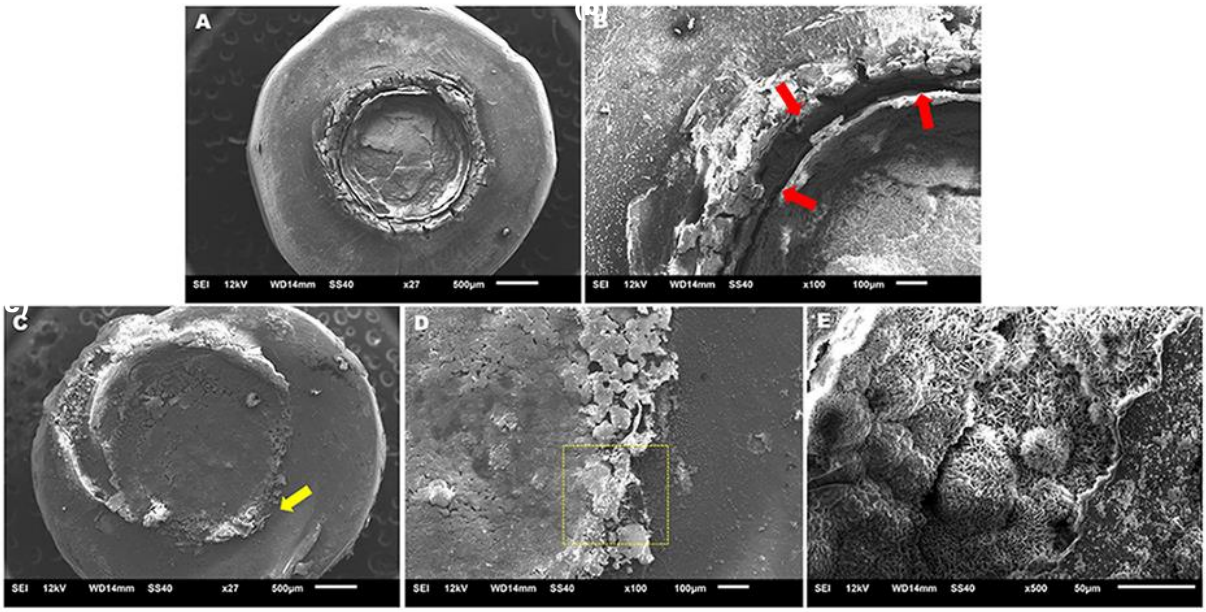

Figure 5 - Scanning Electron Microscopy Images. Dentin tube filled with bioceramic repair material after immersion in butyric acid for 28 days – 27x magnification (A) and (B) formation of cracks in the material/dentin interface (red arrows) – 100x magnification. Dentin tube filled with bioceramic repair material after immersion in PBS – 27x magnification (C); higher magnification (yellow square) demonstrates adaptation in the material/dentin interface - 100X magnification (D) and higher magnification demonstrates formation of hydroxyapatite crystals on the surface at the interface - 500X magnification (E).

4 DISCUSSION

The presence of inflammation provides an acidic pH in the periradicular tissues and can compromise the physicochemical properties of hydraulic repair materials (Namazikhah et al., 2008; Ashofteh et al., 2019; Akinci et al., 2020). This study evaluated the effect of immersion time in butyric acid or PBS on the physical properties of Clinker Angelus, simulating a clinical scenario using a dentin tube model.

The current findings showed that immersion in butyric acid causes greater volumetric loss, porosity, and gaps in the material/dentin interface for all materials evaluated compared to immersion in PBS, rejecting our first null hypothesis. The pH value of a periapical lesion depends on the host´s inflammatory response and bacterial byproducts (Siqueira Jr & Rôças, 2022). However, the presence of an acidic environment impairs hydration and the formation of carbonated hydroxyapatite in bioceramic cements (Akinci et al., 2020). Furthermore, butyric acid (pH 4.4) has been

shown to significantly reduce the surface microhardness of White ProRoot MTA (Namazikhah et al., 2008). Based on these findings, the present study used butyric acid (pH 4.1) to mimic the presence of an acidic pH in the periradicular region, considering that it is one of the metabolic byproducts of anaerobic bacteria present in inflammation (Eftimiadi et al., 1987).

When evaluating the impact of pH on material properties, our results revealed that all experimental groups had similar volumetric stability to BCR and BIO regardless of the immersion solutions and evaluation period. Long-term dimensional stability favors sealing (Torres et al., 2020b). BCR and BIO showed volumetric stability after immersion in distilled water or PBS (Torres et al, 2020a; Torres et al., 2021; Campi et al., 2023). On the other hand, volumetric loss was observed for BIO and BCR when exposed to an acidic environment (Akinci et al., 2020, Silva et al., 2024). However, as this is the first study evaluating the volumetric behavior of clinker after acid challenge, direct comparisons with other reports were not possible.

Rejecting the second null hypothesis, CL<2+ ZrO_2+DW showed greater volumetric loss compared to CL2 to 30+ZrO_2+LA after 7 and 28 days, considering the extremities of the samples (Figure 2). The greatest volume loss may be related to particle size and handling with distilled water. The hydration of bioceramic materials with smaller particles provides greater contraction of the material during setting (Bentz, Garboczi, Haecker & Jensen, 1999), compromising its sealing capacity. Furthermore, the rapid dissolution of smaller particles in the presence of water accelerates the material's setting reaction (Ha et al., 2015). Our results also indicate that the BCR and CL< 2 groups manipulated with DW or LA showed volumetric loss after 7 days. The presence of plasticizer agents in the liquid of hydraulic materials can cause greater mass loss (Guimarães et al., 2018). Furthermore, the surfactant effect of the plasticizer causes the dispersion of cement particles (Dawood et al., 2015) and may be correlated with the volume loss of the materials.

Low porosity and adequate sealing in the material/dentin interface reduce the possibility of bacterial infiltration (Inada et al., 2023). CL2 to 30+ZrO_2+DW demonstrated greater porosity and greater gaps in the material/dentin interface compared to the other groups in this study (Figure 3). The incomplete hydration of larger cement particles provides a greater presence of voids due to the scarce participation of hydration products (Torres, Guerreiro-Tanomaru, Bosso-Martelo,

Chavez-Andrade & Tanomaru Filho, 2018; Camilleri et al., 2014). A high porosity can affect the stability, integrity, and durability of repair materials (Mutal & Gani, 2005). Furthermore, our results showed that BIO presented high porosity at the extremities and internal part of the samples (Figure 3). Conversely, previous studies observed lower porosity for BIO when immersed in PBS or butyric acid (Akinci et al., 2020; Torres et al., 2020a). This difference may be related to the powder-liquid ratio used in the present study since the increase in liquid is correlated with the greater porosity of the material (Camilleri et al., 2014). On the other hand, BCR showed lower porosity, but a higher percentage of gaps in the material/dentin interface after immersion in butyric acid for 7 and 28 days compared to PBS. In contrast, a low percentage of gaps in the material/dentin interface was reported for BCR after implantation in rat subcutaneous tissue (Inada et al., 2023). Possibly, these divergent results are related to the different methodologies used, such as the tissue fluid used, the pH of the solution, and samples contact with the materials.

The period of immersion in butyric acid for 28 days was shown to influence the volumetric change, porosity, and material/dentin interface in relation to the period of 7 days. In agreement, the literature also correlated an increase in volume loss and a higher percentage of gaps in the material/dentin interface for calcium silicate-based cements after 30 days and 60 days, respectively (Inada et al., 2023; Campi et al., 2023). Therefore, the third null hypothesis was rejected. Longer periods allow a better understanding of the behavior of bioceramic materials, due to the hydration process continuing even after the final setting time (Siboni, Taddei, Zamparini, Prati & Gandolfi, 2017). It is important to highlight that this is the first study to evaluate the effects of butyric acid and PBS on the volumetric change, porosity, and gaps in the material/dentin interface of repair bioceramic materials after 28 days and could be a starting point for future investigations.

According to the analysis of the material surface using SEM, structural loss, and corrosion of the material, as well as cracks at the material/dentin interface, were observed in butyric acid. This finding reinforces the premise that an acidic pH interferes with the hydration of the material and reduces the deposition of apatite on the surface and at the material/dentin interface (Akinci et al., 2020). On the other hand, the formation of hydroxyapatite crystals on the materials surface and tag formation at the material/dentin interface was observed when immersed in PBS (Figure 5), in

agreement with previous studies (Reyes-Carmona et al., 2009; Reyes-Carmona, Felippe & Felippe, 2010), suggesting a bioactive potential for these materials. The association of SEM and micro-CT analyses can be considered powerful approaches to evaluating the effect of different immersion solutions on the physical behavior of hydraulic materials.

Regarding the methodology adopted, bovine dentin tube models were used since they allow the interaction of bioceramic materials with the dentin walls (De-Deus et al., 2022), approaching a real clinical condition (Inada et al., 2023). Furthermore, analyses in different parts of the specimen (extremities and internal part – Figure 1) allowed monitoring of the behavior of materials inside the mass and those close to the tissue fluid (Inada et al., 2023). Besides, evaluation of material properties at different periods was carried out using high-resolution micro-CT, facilitating the measurement of the volume and difference between the filling material, voids, and tooth structure (Celikten et al., 2015). Micro-CT is a high-precision methodology and is considered the gold standard for investigating the physical and morphological properties of endodontic materials (Celikten et al., 2015; Torres et al., 2018; Akinci et al. 2020; Torres et al., 2020a; Torres et al. 2020b; Torres et al., 2021; Vergaças et al., 2022; Inada et al., 2023; dos Santos et al., 2024). Although Biodentine is considered a comparison material due to its adequate physicochemical properties (Campi et al., 2023), it was not possible to perform gaps analysis in the material/dentin interface for Biodentine. As it is a material with low radiopacity (2.30 mm Al) (Campi et al., 2023), its similarity with the radiopacity of root dentin (2.27 mm Al) (Yaylacı, Karaarslan & Hatırlı, 2021) can difficult the segmentation process of micro-CT images (De-Deus et al., 2022).

Knowledge of the properties of repair materials is important, but their application in different clinical conditions is even more relevant. The results of this study suggest that an acidic pH harms the physical properties of Clinker Angelus, especially when manipulated with distilled water. These findings can help the clinician make the appropriate choice of bioceramic repair material when faced with a periradicular lesion. In addition, knowledge of the behavior of the repair material in an acidic environment can favor the prognosis of long-term treatment. Future *in-vivo* and *in-vitro* studies should be encouraged to evaluate other physicochemical and biological properties of Clinker Angelus under different pH conditions.

5 CONCLUSIONS

Acidic pH increases the gaps in the material/dentin interface, porosity, and the dimensional change for bioceramic materials. The manipulation of the Clinker with distilled water presented the highest porosity and interface gaps, as well as the greatest volume loss.

Conflict of interest disclosure

The authors declare there are no conflicts of interest regarding this article.

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3.3 Publicação 3*

Effects of acidic pH and sealer thickness on the physical properties of endodontic sealers. Micro-computed tomographic study

ABSTRACT

The aim of this study was to evaluate the effect of immersion in an acidic (butyric acid – BA; pH 4.1) or neutral (phosphate buffered saline **-** PBS; pH 7.0) environment on the physical behavior of Bio-C Sealer (BCS) or AH Plus Jet (AHPJ) after filling oval root canals using different sealer thicknesses. Thirty-two oval root canals were prepared and obturated using the single cone technique with BCS or AHPJ (n=16). Greater, middle, and smaller thickness of sealer was established in the cervical, middle, and apical thirds of the root canals, respectively. Then, the samples were inserted individually into the Eppendorf tube with 1.5 mL of BA or PBS (n=8) for 7 and 30 days. Scans using micro-computed tomography (micro-CT) after the materials set and after experimental periods were performed. Kruskal-Wallis e Dunn, Friedman, Wilcoxon paired, and ANOVA e Tukey tests were performed (α=0.05). Volumetric change and the percentage of voids and gaps in the material/dentin interface were evaluated. Significant volumetric change and percentage of voids and gaps in the interface of BCS and AHPJ were observed in an acid environment after 30 days ($p<0.05$). Both materials showed a high percentage of voids and volumetric loss in the greater sealer thickness ($p<0.05$). Baseline showed a lower percentage of voids and gaps in the interface when compared to the experimental periods ($p<0.05$). An acidic environment harms the properties of endodontic sealers after 30 days. The greater sealer thickness negatively affects the volumetric change and presence of voids in oval root canals.

Key Words: dental materials, micro-computed tomography, physical process, root canal obturation

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Introduction

Three-dimensional filling of root canals is essential, especially in root canals with anatomical complexities (1, 2). Among the obturation techniques, the single cone technique is considered easy to perform (3), but a greater amount of sealer is used for adequate filling of the root canals (3, 4). Therefore, the properties of endodontic sealers are even more important since their volumetric behavior, filling capacity, and interaction with dentin can impact the success of endodontic treatment (1, 2, 4-7).

Bio-C Sealer (BCS, Angelus, Londrina, PR, Brazil) is a pre-mixed, ready-to-use sealer with properties achieving the ISO 6876:2012 requirements (5), including setting time, radiopacity and flow, alkaline pH, and low volumetric change by micro-computed tomographic analysis (micro-CT) (8). Furthermore, BCS showed a low percentage of voids similar to AH Plus (AHP; Dentsply DeTrey GmbH, Konstanz, Germany) (2), besides a low presence of marginal gaps when compared to AH Plus Jet (AHPJ; Dentsply) (7). However, BCS does not follow the solubility values stipulated by ISO 6876 (5, 8, 9). AHPJ is an epoxy resin-based sealer available in paste-paste form in an automix syringe. This material is usually used as a comparison standard for investigating new sealers (2, 8, 10) due to its excellent physicochemical properties (8, 10), including low solubility (9, 10).

The physicochemical behavior of endodontic sealers can be affected in an acidic environment (11-13). Acidic pH derived from the by-products of inflammation in the presence of apical periodontitis has been shown to change the chemical composition and damage the hydration reaction of ProRoot MTA cement (Dentsply Maillefer, Ballaigues, Switzerland), Biodentine (Septodont, Saint-Maur-des-Fossés, France), CEM Cement (Bionique, Tehran, Iran) and Retro MTA (BioMTA, Seoul, Republic of Korea) (12). Furthermore, acidic pH increases the volumetric loss of Biodentine (11) and EndoSequence BC Sealer (Brasseler, Savannah, GA, USA) (1), and promotes lower bond strength for EndoSequence Root Repair Material (Brasseler) (13). On the other hand, the use of simulated body fluid (phosphate buffered saline - PBS) has been shown to decrease the solubility and volumetric change of calcium silicate-based materials (1, 5, 9). Furthermore, its similarity with a clinical application can help to understand the physical behavior of root canal sealers (5, 9).

Sealer thickness is another factor that influences the quality of the root canal filling (4, 14). A smaller thickness of sealer between the gutta-percha and the root canal

wall is considered ideal (3). Generally, the cervical third provides a greater amount of sealer during filling (4, 14), especially in root canals with larger buccolingual extensions (2, 4, 7). Higher percentage of voids in the root canal filling for BioRoot RCS compared to GuttaFlow Bioseal (Coltene Whaledent, Langenau, Germany) was observed in the cervical third of the root canals (4). Furthermore, a higher percentage of voids in the cervical/middle third was observed when using the single cone technique in comparison to Tagger´s hybrid technique (15). On the other hand, a low percentage of voids has been reported for flattened root canals when obturated with the single cone technique using BCS or AHP (2). Until now, there are still no studies in the literature that evaluate the influence of sealer thickness on the behavior of endodontic materials in different pH conditions.

The aim of this study was to evaluate the effect of immersion in an acidic (butyric acid – BA) or neutral (PBS) environment on the physical behavior of BCS or AHPJ after filling oval canals using different sealer thicknesses through micro-CT. The first null hypothesis was that the immersion solution would not influence the volumetric change, percentage of voids, and gaps in the material/dentin interface after filling oval root canals using BCS or AHPJ. The second null hypothesis was that the sealer thickness would not interfere the properties of the materials and the third hypothesis was that there would be no difference between the variables in relation to the immersion period in BA or PBS for both sealers.

Material and methods

Sample size calculation

The G*Power 3.1.7 program for Windows (Heinrich-Heine-Universitat Dusseldorf, Dusseldorf, Germany) was used for sample calculation. One-Way ANOVA test was used with an alpha-type error of 0.05 and a beta power of 0.85 for all the variables. Previous studies were used to determine the specific effect size for volumetric change, 0.98 (16), percentage of voids, 0.71 (17), and gaps in the interface, 1.76 (18). A total of 8 specimens were indicated as the ideal size required.

Sample selection

After approval by the Institutional Research Ethics Committee (Protocol CAAE No. 41926620.6.0000.5416), 32 extracted human teeth with a fully formed apex, absence of fractures, and internal resorption were selected. The teeth had oval root canals previously selected by a digital radiography system (Kodak RVG 6100; Digital Radiography System, Marne-la-Vallée, France) and micro-CT with a voxel size of 35 µm (SkyScan 1272, Bruker-microCT, Kontich, Belgium). The oval cross-section of the root canals was determined when the buccolingual diameter was equal to 2 or more times larger than the mesiodistal diameter (19) at 9 mm from the radiographic apex (2), using the CTAn software (v.1.14.4, SkyScan, Belgium). Teeth with an immature apex, curvatures, and previous endodontic treatment were excluded.

Root canal preparation

After coronary access, the root canals were explored with a size 15 and 20 K-file (Dentsply, Maillefer, Ballaigues, Switzerland). The working length (WL) was determined to be 1 mm beyond the apical foramen. The roots were molded with condensation silicone (Oranwash, Zhermack SpA, Badia Polesine, Italy) to simulate the periodontal ligament and stabilize the samples during the procedures (2, 15). Subsequently, a single operator previously trained and calibrated prepared the root canals using the ProDesign Logic rotary system (Easy Equipamentos Odontológicas, Belo Horizonte, MG, Brazil) 40/.05 at 950 RPM and 4Ncm driven by a VDW SILVER electric motor. (VDW GmbH, Munich, Germany). During the entire preparation, the canals were irrigated with 5 mL of 1% sodium hypochlorite (NaOCl; Ciclo Farma, Serrana, SP, Brazil) using a 5 mL syringe (Ultradent, South Jordan, UT) with a 30G Navitip needle (Ultradent). After preparation, the 10 millimeters of the apical roots were sectioned using an Isomet 1000 (Buehler Ltd., Lake Bluff, Illinois, United States). Subsequently, the final irrigation of the specimens was performed with 5 mL of 2.5% NaOCl and 5 mL of 17% EDTA (Biodynamics, Ibiporã, PR, Brazil) for 3 minutes, followed by 5 mL of distilled water.

Root canal filling

The root canals were filled using the single-cone technique. 40/.04 gutta-percha master cones (Tanariman, Manacapuru, AM, Brazil) were selected according to tip size

and taper by a Profilometer device (Profile Projector Nikon model 6C-2, Nikon, Tokyo, Japan). Digital radiographs were taken to confirm the adaptation of the cone at 1 mm beyond the apical foramen. BCS or AHPJ sealers (n=16) were manipulated following the manufacturer's recommendations (Table 1) and inserted into the root canal according to a previous study (2). The thickness of the sealer was determined from the thirds of the root canal (3 mm each), obtaining a smaller thickness in the apical third, a middle thickness in the middle third, and a greater thickness in the cervical third (Figure 1A). After insertion of the gutta-percha cone coated with sealer inside the canal, the excess of gutta-percha was cut and compacted vertically with a heat plugger (Golgran nº 2, São Caetano do Sul, Brazil). Digital radiographs in the buccolingual and mesiodistal directions were taken to evaluate the quality of root canal filling. The samples were stored in an oven at 37º C and 95% humidity for 7 days to allow complete setting of the sealers.

Table 1 – Experimental groups, material with their manufacturers, proportion, sealer thickness and immersion solutions

Groups	Material and manufacturers	Proportion	Sealer thickness	Immersion solutions
BCS BA or PBS	Bio-C Sealer Angelus, Londrina, PR, Brazil Lot: 64361	Ready to use	Greater thickness: 3 mm Cervical third	Butyric acid PBS
BCS BA or PBS	Bio-C Sealer Angelus, Londrina, PR, Brazil Lot: 64361	Ready to use	Middle thickness: 3 mm Middle third	Butyric acid PBS
BCS BA or PBS	Bio-C Sealer Angelus, Londrina, PR, Brazil Lot: 64361	Ready to use	Smaller thickness: 3 mm Apical third	Butyric acid PBS
AHPJ BA or PBS	AH Plus Jet Dentsply DeTrey GmbH, Konstanz, Germany Lot: 2205000392	1g:1g (paste/paste) automix	Greater thickness: 3 mm Cervical third	Butyric acid PBS
AHPJ BA or PBS	AH Plus Jet Dentsply DeTrey GmbH, Konstanz, Germany Lot: 2205000392	1g:1g (paste/paste) automix	Middle thickness: 3 mm Middle third	Butyric acid PBS
AHPJ BA or PBS	AH Plus Jet Dentsply DeTrey GmbH, Konstanz, Germany Lot: 2205000392	1g:1g (paste/paste) automix	Smaller thickness: 3 mm Apical third	Butyric acid PBS

BCS: Bio-C Sealer, **AHPJ:** AH Plus Jet, **BA:** Butyric acid, **PBS:** Phosphate buffered saline

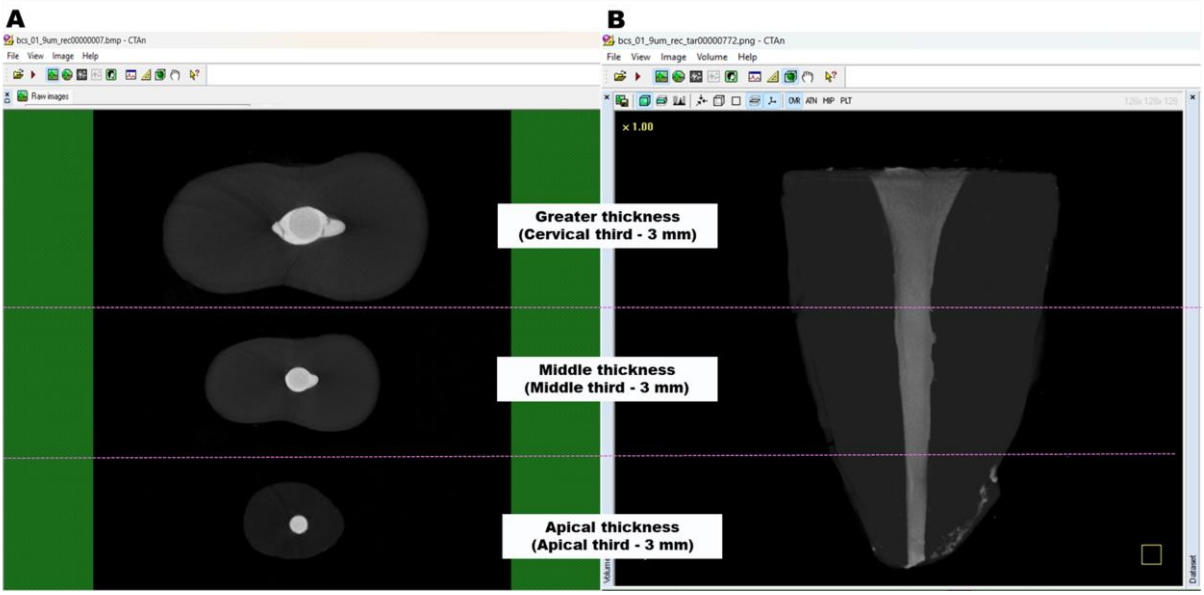

Figure 1 – Representative image of the sample division for analysis using the CTAn software. (*A*) Sealer Thickness and (*B*) thirds of the oval root canals

Micro-CT scanning and immersion of the specimens

After obturation, all samples were scanned in micro-CT (SkyScan 1272, Bruker, Kontich, Belgium) using the following parameters: voxel size of 8.74 µm, rotation step 0.5, frame averaging of 3, Al filter 0.5 + Cu.038, rotation 180°, 90 kV and 110 uA. Then, each specimen was inserted individually into the Eppendorf tube with 1.5 mL of BA (Sigma Aldrich, Barueri, SP, Brazil, BA; pH: 4.1) or PBS (Sigma, pH: 7.0) (n=8) and stored in an oven at 37 ºC for 7 and 30 days. The BA solution was changed every 24 hours to ensure a stable acidic pH throughout the experiment. New micro-CT scans were conducted after the different experimental periods using the same parameters described previously.

Micro-CT analysis

Image reconstructions in the different experimental periods were performed using the NRecon software (v.1.6.3; Bruker). 3D images were registered before and after immersion using Data Viewer software (v.1.5.1.; Bruker). Quantitative analyses were performed using the CTAn software (v.1.15.4.0; Bruker). Representative images were created using the CTVOx software (v.3.2; Bruker). Assessments were performed for each third of the specimen according to the filling material and sealer thickness:

smaller thickness (3 mm from the apical third), middle thickness (3 mm from the middle third), or greater thickness (3 mm from the cervical third) (Figure 1B).

The volumetric change was determined by calculating the difference in the total volume of the materials, in mm^3, before and after immersion in BA or PBS for 7 and 30 days. The percentage of voids was determined considering the percentage of filling material (sealer and gutta-percha) after the sealer set and after experimental periods, using the following formula: [Percentage of voids = 100 - percentage of filling material]. The percentage of gaps in the material/dentin interface was calculated based on the methodology described by Gandolfi et al. (20). The 3D distribution of gaps in the interface with a size starting at 8.74 µm within a pre-defined volume of interest (VOI), including part of the dentin and part of the filling material, was detected using a ¨*task list*¨ with arithmetic and logical operations between the superimposed sections. All analyses were carried out using a grayscale to identify each study object using a density histogram with adaptive thresholding.

Statistical Analysis

All data were subjected to the Shapiro-Wilk test. Data relative to volumetric change and percentage of voids presented a non-normal distribution. Kruskal-Wallis and Dunn tests were used to compare groups. The Friedman and Wilcoxon Paired tests were used to compare the immersion periods. Data relative to the percentage of gaps in the material/dentin interface showed a normal distribution. One- way ANOVA and Tukey tests were used for comparisons between groups. Repeated measures ANOVA and paired Tukey were used for comparisons among immersion periods. The level of significance was 5% for all analyses.

Results

In the period of 7 days, BCS and AHPJ showed similar volumetric changes and percentages of gaps in the interface regardless of the immersion solution and sealer thickness ($p>0.05$). After 30 days, BA increased the volumetric loss and presence of voids and gaps in the material/dentin interface of both materials ($p<0.05$) (Tables 2, 3, and 4).

Regarding sealer thickness, a higher presence of voids was observed in the greater thickness compared to the smaller thickness in both sealers after 7 days

($p<0.05$) (Figure 3). Immersion in BA for 30 days resulted in greater volume loss in the greater and smaller thicknesses for both materials compared to the middle thickness ($p<0.05$) (Figure 2). Furthermore, BCS showed a higher percentage of gaps in the material/dentin interface in the greater thickness compared to the middle thickness ($p<0.05$). The greater sealer thickness for AHPJ showed a higher percentage of voids compared to the smaller thickness without the influence of the immersion solution after 30 days ($p<0.05$) (Figure 3).

Concerning the immersion period, higher volumetric loss was reported for AHPJ in the greater and smaller thickness, and for BCS in the greater thickness after 30 days in BA than 7 days ($p<0.05$) (Figure 2, Table 2). Baseline showed a lower percentage of voids and gaps in the interface when compared to the immersion periods of 7 and 30 days ($p<0.05$) (Figure 3).

Table 2 - Median, minimum, and maximum values of the percentage of volumetric change of Bio-C Sealer or AH Plus Jet after immersion in butyric acid (BA) or PBS for 7 and 30 days

	Immersion period 7 days			
	Bio-C Sealer		AH Plus Jet	
	BA	PBS	BA	PBS
GT Cervical third	-1.23 (-3.36-(-0.58))^{aA*}	-0.36 (-2.40-1.40)^{aA}	-0.88 (-4.54-0.28)^{aA*}	1.27 (-2.90-2.67)^{aA}
MDT Middle third	-0.21 (-3.31-0.24)^{aA}	-0.04 (-1.71-1.62)^{aA}	-1.10 (-1.07-(-0.04))^{aA}	-0.99 (-2.63-1.31)^{aA}
ST Apical third	-2.00 (-9.93-(-0.26))^{aA}	-0.28 (-3.97-0.94)^{aA}	-2.44 (-4.20-0.45)^{aA*}	-1.17 (-3.09-1.14)^{aA}
	Immersion period 30 days			
	Bio-C Sealer		AH Plus Jet	
	BA	PBS	BA	PBS
GT Cervical third	-4.58 (-5.92-(-2.87))^{bB*}	-0.77 (-3.05-1.11)^{aA}	-3.77 (-10.46-(-2.28))^{bB*}	-1.59 (-4.89-1.91)^{aA}
MDT Middle third	-1.22 (-3.80 – 0.89)^{abA}	-0.83 (-2.44-(1.20))^{bA}	-1.72 (-4.27-(-0.97))^{aA}	0.15 (-3.54-5.69)^{abA}
ST Apical third	-3.30 (-11.78-(-0.35))^{bB}	1.78 (-5.76-2.96)^{aA}	-4.00 (-5.39-(-1.64))^{bB*}	-0.55 (-4.70-3.10)^{abA}

GT: Greater thickness, MDT: middle thickness, ST: smaller thickness.
Negative values: volumetric loss, Positive values: volumetric gain
Different superscript lowercase letters in the same line indicate a significant difference between groups ($P < .05$). Different superscript uppercase capital in the same column indicates a significant difference between the thirds of each group ($P < .05$). *indicates significant difference between immersion periods ($P < .05$).

Table 3 - Median, minimum, and maximum values of the percentage of voids of Bio-C Sealer or AH Plus Jet before and after immersion in butyric acid (BA) or PBS for 7 and 30 days

	Initial period			
	Bio-C Sealer		AH Plus Jet	
	BA	PBS	BA	PBS
GT Cervical third	0.64 (0.09-1.92)^{bAB*}	0.33 (0.13-5.78)^{bA*}	2.30 (0.20-14.77)^{aA*}	1.88 (0.05-6.95)^{abA}
MDT Middle third	0.30 (0.01-0.82)^{aB}	0.47 (0.01-0.78)^{aA}	0.77 (0.01-6.02)^{aAB*}	0.60 (0.01-4.64)^{aAB}
ST Apical third	0.89 (0.07-1.98)^{aA*}	0.25 (0.06-0.78)^{bA}	0.26 (0.06-0.79)$^{bC*\#}$	0.35 (0.01-2.70)^{bB*}
	Immersion period 7 days			
	Bio-C Sealer		AH Plus Jet	
	BA	PBS	BA	PBS
GT Cervical third	1.26 (0.69-2.20)^{aA*}	0.59 (0.13-5.80)^{aA}	2.64 (0.23-6.77)$^{aA\#}$	1.82 (0.23-7.16)^{aA}
MDT Middle third	0.28 (0.06-0.99)^{aB}	0.56 (0.17-1.81)^{aA}	0.98 (0.02-5.23)$^{aAB\#}$	0.16 (0.02-4.79)^{aA}
ST Apical third	1.20 (0.55-1.85)$^{aC\#}$	0.39 (0.09-3.60)^{aA}	0.59 (0.12-1.32)^{aB*}	0.58 (0.02-2.91)^{aA*}
	Immersion period 30 days			
	Bio-C Sealer		AH Plus Jet	
	BA	PBS	BA	PBS
GT Cervical third	3.37 (2.02-9.38)^{aA*}	0.92 (0.22-6.65)^{bA*}	5.53 (0.36-8.18)$^{aA*\#}$	2.31 (0.01-9.21)^{abA}
MDT Middle third	0.39 (0.10-2.17)^{bB}	0.71 (0.03-1.93)^{bA}	2.26 (0.10-6.02)$^{aAB*\#}$	0.58 (0.01-4.82)^{bAB}
ST Apical third	1.98 (1.24-5.16)$^{aA*\#}$	0.24 (0.01-2.96)^{bA}	1.24 (0.11-1.99)$^{abB\#}$	0.41 (0.07-3.61)^{cB}

GT: Greater thickness, MDT: medium thickness, ST: smaller thickness. Different superscript lowercase letters in the same line indicate a significant difference between groups ($P < .05$). Different superscript uppercase letters in the same column indicate a significant difference between the thirds of each group ($P < .05$). Similar symbols indicate significant difference between immersion periods. ($P < .05$).

Table 4 - Mean and ±standard deviation of the percentage of gaps in the material/dentin interface of Bio-C Sealer and AH Plus Jet before and after immersion in butyric acid (BA) or PBS for 7 and 30 days

	Initial period			
	Bio-C Sealer		AH Plus Jet	
	BA	PBS	BA	PBS
GT Cervical third	5.77 ± 2.19^{aA*}	5.67 ± 2.23^{aA}	7.69 ± 2.53^{aA*}	8.55 ± 3.16^{aA}
MDT Middle third	4.68 ± 2.09^{aA*}	4.76 ± 2.14^{aA}	7.07 ± 2.16^{aA*}	6.86 ± 2.39^{aA}
ST Apical third	5.73 ± 2.10^{aA*}	4.63 ± 2.03^{aA*}	6.54 ± 1.54^{aA*}	6.13 ± 1.60^{aA*}
	Immersion period 7 days			
	Bio-C Sealer		AH Plus Jet	
	BA	PBS	BA	PBS
GT Cervical third	7.16 ± 2.25^{aA*}	6.33 ± 2.30^{aA}	9.27 ± 3.94^{aA*}	9.79 ± 3.43^{aA}
MDT Middle third	5.93 ± 2.06^{aA*}	5.47 ± 2.28^{aA}	8.34 ± 2.48^{aA}	8.30 ± 2.38^{aA}
ST Apical third	6.93 ± 2.34$^{aA\#}$	5.62 ± 1.87^{aA}	8.12 ± 1.75^{aA*}	7.27 ± 1.98^{aA}
	Immersion period 30 days			
	Bio-C Sealer		AH Plus Jet	
	BA	PBS	BA	PBS
GT Cervical third	10.03 ± 2.85^{abA*}	6.42 ± 1.97^{bA}	11.89 ± 4.04^{aA*}	10.29 ± 4.73^{abA}
MDT Middle third	6.92 ± 1.76^{aB*}	5.59 ± 2.18^{aA}	9.18 ± 2.87^{aA*}	7.84 ± 3.61^{aA}
ST Apical third	8.90 ± 2.13$^{aAB*\#}$	5.82 ± 1.88^{bA*}	9.70 ± 1.85^{aA*}	7.94 ± 1.96^{abA*}

GT: Greater thickness, MDT: medium thickness, ST: smaller thickness. Different superscript lowercase letters in the same line indicate a significant difference between groups ($P < .05$). Different superscript uppercase letters in the same column indicate a significant difference between the thirds of each group ($P < .05$). Similar symbols indicate significant difference between immersion periods. ($P < .05$).

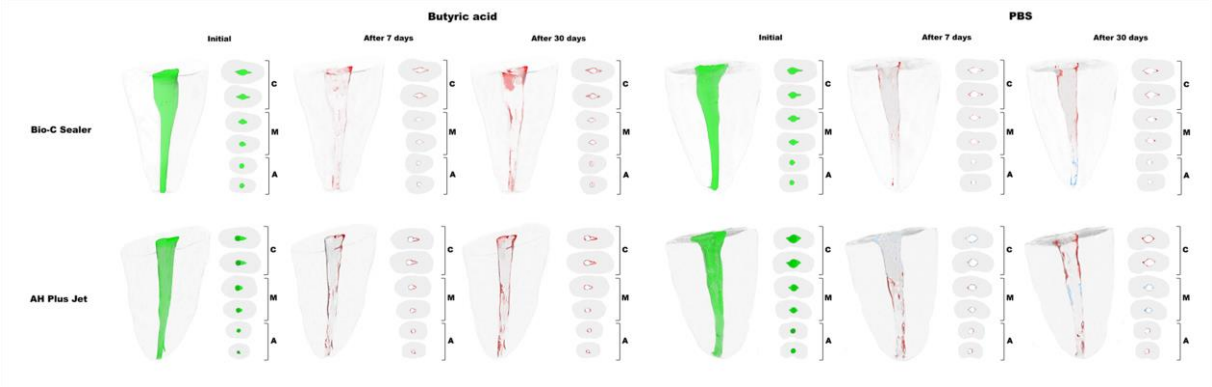

Figure 2 – Three-dimensional images and cross-sectional views of the cervical (C), middle (M), and apical (A) thirds of 4 oval root canals showing the initial volume (*green*), volume gain (*blue*) and volume loss (*red*) of the Bio-C Sealer or AH Plus Jet before and after immersion in butyric acid or PBS for 7 and 30 days

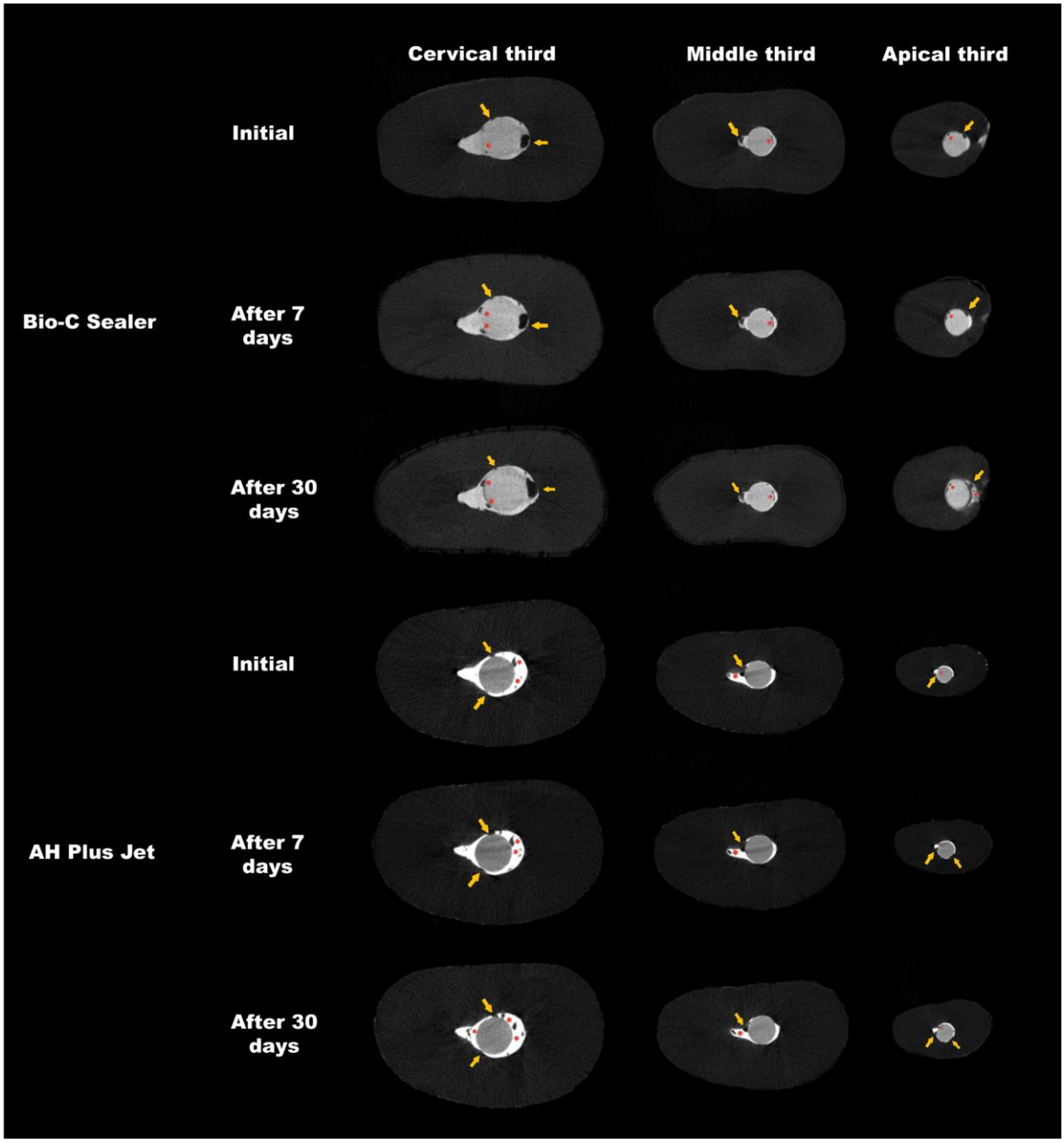

Figure 3 – Representative cross-sectional views of 2 oval root canals showing the voids (*red asterisks*) and gaps in the dentin/material interface (*yellow arrows*) of Bio-C Sealer or AH Plus Jet before and after immersion in butyric acid for 7 and 30 days

Discussion

The sealer thickness between the gutta-percha and dentin must be as small as possible since this material is subject to solubilization and dimensional changes (3). Furthermore, the presence of an acidic pH in the periradicular tissues and infection inside the root canals can compromise the root canal sealing (1, 11-13). Therefore, the present study was conducted to evaluate the effect of immersion in an acidic or neutral

environment after 7 and 30 days, as well as the influence of sealer thickness on the physical properties of the BCS or AHPJ.

The first null hypothesis was rejected. Significant volumetric loss and a higher percentage of voids and gaps in the material/dentin interface were observed for both materials after exposure to acidic pH for 30 days. These results may be related to the inhibition of the hydration and setting reactions of these materials, and less deposition of carbonated apatite on the surface and interface of the material/dentin. The hydrophilic particles of calcium silicate-based sealers have greater contact with liquid molecules (21). Thus, the increase in water sorption (absorption and adsorption of liquids) and solubility (loss of mass) can affect the dimensional stability of materials (22) and reduce the filling capacity (23). Furthermore, structural changes in hydroxyapatite crystals after acid challenge cause greater porosity and solubility for bioceramic materials (1). The corrosive effect of the acidic environment and the exposure time may also explain the results observed for the degradation of the AHPJ sealer (24).

When evaluating the sealer thickness on the properties of BCS or AHPJ, our results indicated that the greater thickness of both sealers negatively affects the properties evaluated regardless of the period, rejecting the second null hypothesis. Root canals have a flattened cross-section in the cervical third (2). Thus, greater sealer thickness may favor the presence of voids and gaps in the dentin/material interface. In agreement with our findings, lower filling capacity in the cervical third was observed when calcium silicate-based sealers were used (4). However, contrary to our results, a previous study showed a lower percentage of voids for BCS and AHPJ, besides a low percentage of gaps in the interface for BCS after filling oval root canals using the continuous condensation wave technique or the Tagger's hybrid technique (7). This difference is possibly related to the obturation technique used in the present study. The single cone technique requires a greater amount of sealer (2, 3), which may negatively affect the volumetric behavior of the material. Furthermore, the hydrophobic nature of gutta-percha may negatively affect its interaction with the material, considering the hydrophilic nature of calcium silicate-based sealers (6). Overall, these findings could explain the volume loss and higher percentage of voids and gaps in the interface observed in the oval root canals in this study. On the other hand, the greater thickness of the AHPJ sealer presented a higher percentage of voids compared to the smaller

thickness, regardless of the immersion solutions. This result may be related to the hydrophobic nature of the AHPJ, which can interfere with the behavior of the material in canals with larger buccolingual extensions. Disagreeing with our findings, a previous report showed a low percentage of voids in the material/gutta-percha interface when using AHPJ after immersion in PBS for 7 days (6). The divergence of these results is possibly related to the methodology used since prolonged periods of immersion were employed in the present study (7 and 30 days).

Longer periods of assessment of the physical properties of materials are essential, considering that the hydration reaction continues even after the final setting time (21). A previous study showed higher volumetric loss and a lower percentage of voids for BCS when immersed in distilled water for 7 days (5). In the present study, higher volumetric loss and a higher percentage of voids and gaps in the material/dentin interface of BCS and AHPJ were observed over a period of 30 days in comparison to the 7 days, leading to the rejection of the third null hypothesis. This finding may be related to the acidic environment and the prolonged period of exposure. In agreement with our results, a previous study reported greater volumetric loss for EndoSequence BC Sealer after 30 days in acidic pH (1).

The quality of the filling is influenced by the morphology of the root canals (2, 6). In this study, root canals with an oval cross-section were used as they represent a real challenge for adequate filling in clinical practice (1, 6, 7). Furthermore, the variables could be quantified through of analyses using high-resolution micro-CT images in relation to volumetric behavior and the presence of voids and gaps in the material/dentin interface of endodontic sealers after the different experimental periods of the present study (1-8, 11, 15-17). In order to simulate a scenario close to clinical, BA was used, as it represents one of the metabolic by-products of anaerobic bacteria present in inflammation (25). PBS was considered a comparison parameter as it mimics a neutral environment (1, 5, 9). However, as it is a laboratory study, *in-vivo* studies should be encouraged to offer more evidence that allows the correct use of these materials in different clinical situations.

Within the limitations of this *ex-vivo* study, it can be concluded that an Acidic environment impairs the properties of Bio-C Sealer and AH Plus Jet, especially after immersion for 30 days. The greater sealer thickness in the cervical third negatively affects the quality of the filling for both sealers.

DECLARATION OF CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

Abstract in Portuguese

O objetivo deste estudo foi avaliar o efeito da imersão em ambiente ácido (ácido butírico – AB; pH 4,1) ou neutro (solução salina tamponada com fosfato – PBS; pH 7,0) no comportamento físico do Bio-C Sealer (BCS) ou AH Plus Jet (AHPJ) após obturação de canais radiculares ovais com diferentes espessuras de cimento. Trinta e dois canais radiculares ovais foram preparados e obturados pela técnica de cone único com BCS ou AHPJ (n=16). Espessura maior, média e menor de cimento foram estabelecidas nos terços cervical, médio e apical dos canais radiculares, respectivamente. Em seguida, as amostras foram inseridas individualmente em tubo Eppendorf com 1,5 mL de AB ou PBS (n=8) por 7 e 30 dias. Escaneamentos em microtomografia computadorizada (micro-CT) após presa dos materiais e após os períodos experimentais foram realizados. Testes de Kruskal-Wallis e Dunn, Friedman, Wilcoxon pareados e ANOVA e Tukey foram realizados (α=0,05). Alteração volumétrica e porcentagem de vazios e falhas na interface material/dentina foram avaliados. Significativa alteração volumétrica e porcentagem de vazios e falhas na interface do BCS e AHPJ foram observadas em ambiente ácido após 30 dias ($p<0,05$). Ambos materiais apresentaram alto porcentagem de vazios e perda volumétrica na espessura maior de cimento ($p<0,05$). Baseline apresentou menor porcentagem de vazios e falhas na interface quando comparado aos períodos experimentais ($p<0,05$). Ambiente ácido prejudica as propriedades dos cimentos endodônticos após 30 dias. A espessura maior de cimento afeta negativamente a alteração volumétrica e a presença de vazios em canais radiculares ovais.

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4 DISCUSSÃO

O emprego amplo de materiais à base de silicato de cálcio na Endodontia[4] decorre de suas propriedades biológicas e físico-químicas[6,11,38]. O tamanho de partícula[49,50], presença do radiopacificador[11,18] e a manipulação com diferentes líquidos[18,24,46] são fatores que apresentam efeito nas propriedades físicas, químicas e biológicas destes materiais. O Clínquer é a matéria-prima utilizada na fabricação de cimentos reparadores e obturadores da Angelus (Angelus® Indústria de Produtos Odontológicos S/A, Londrina, PR, Brasil). Os resultados da publicação 1 revelaram que o menor tamanho de partícula (<2 µm) proporciona um menor tempo de presa final e uma maior capacidade de alcalinização para o Clínquer Angelus (Tabela 2 e 3, Publicação 1). Estes resultados podem estar relacionados com a maior área de superfície quando um menor tamanho de partícula é usado, proporcionando menor tempo de tempo de presa e maior liberação de hidróxido de cálcio[52,53]. Com relação a associação ao radiopacificador, o tungstato de cálcio propicia adequada radiopacidade sem modificar as demais propriedades, como observado em estudo anterior[18].

Os agentes plastificantes adicionados à água melhoram o manuseio e inserção do material[24]. No entanto, estudos prévios relataram maior solubilidade, porosidade e falhas na interface material/dentina para materiais manipulados com líquido com aditivos[23,24]. A Publicação 1 mostrou que a manipulação do Clínquer com líquido com aditivos apresentou maior radiopacidade e ganho de massa (Tabela 2, Publicação 1) e a Publicação 2 mostrou que o Clínquer quando manipulado com água destilada aumenta a porosidade (Tabelas 4 e 5), as falhas na interface material/dentina (Tabelas 6 e 7), além de promover maior perda de volume (Tabelas 2 e 3). Com base nestes resultados, o emprego de líquido com aditivos apresenta vantagens em relação a água destilada, uma vez que proporciona adequadas propriedades físicas para materiais confeccionados a partir do Clínquer Angelus.

Tendo em vista que materiais reparadores e obturadores podem entrar em contato com ambiente ácido na presença de reações inflamatórias na região periapical[34,58-60], os resultados da Publicação 2 demonstram que o pH ácido prejudica a interface material/dentina, porosidade, e promove maior perda volumétrica para o Clinquer com diferente tamanho de partícula, Bio-C Repair e Biodentine. Os achados da Publicação 3 sugerem maior alteração de volume e maior porcentagem de vazios

no interior do material obturador e na interface material/dentina em canais radiculares ovais preenchidos com Bio-C Sealer e AH Plus Jet após imersão em ácido butírico. Tomados em conjunto, esses resultados indicam que a presença de um pH ácido interfere na hidratação do material e na formação de hidroxiapatita carbonatada[34]. Ao considerarmos o tempo de avaliação, períodos prolongados são importantes, devido ao processo de hidratação de cimentos biocerâmicos continuarem mesmo após o tempo de presa final[81]. Resultados da Publicação 2 e 3 mostraram maior perda volumétrica e maior porcentagem de falhas na interface material/dentina nos períodos de 28 e 30 dias em comparação a avaliação por 7 dias.

A espessura do cimento estabelecida durante a obturação pode exercer efeito na adequada obturação dos canais radiculares. Os resultados da Publicação 3 indicam que a maior espessura de material no terço cervical afeta negativamente a alteração volumétrica e aumenta os vazios em canais radiculares ovais obturados com Bio-C Sealer e AH Plus Jet. A técnica de cone único foi utilizada neste estudo, pois permite realizar comparações diretas entre ambos cimentos visto que esta técnica apresenta menor influência do operador, além de ser de fácil execução[2, 68]. Estudos futuros devem ser conduzidos usando técnicas de obturação termoplásticas pois isso poderia diminuir a porcentagem de vazios e falhas na interface material/dentina[69].

Cimentos à base de silicato de cálcio devem apresentar solubilidade menor que 3% e alteração dimensional inferior a 1,0% em contração ou 0,1% em expansão segundo as normas do *International Organization for Standardization* – ISO e *American National Standards Institute* e *American Dental Association* -ANSI/ADA. No entanto, estas diretrizes não são ideais para avaliação de cimentos à base de silicato de cálcio[61,64]. Neste contexto, a avaliação do comportamento de materiais biocerâmicos usando testes com microtomografia computadorizada (micro-CT) e microscópio eletrônico de varredura (MEV) representam alternativas metodológicas importantes[1,23,30,34,38,43,46,60,62,66-69,78-80]. Na Publicação 2 e 3 as variáveis de alteração volumétrica, porosidade, porcentagem de vazios e falhas na interface material/dentina puderam ser quantificadas volumetricamente em diferentes períodos, afim de fornecer maior entendimento sobre o comportamento de materiais biocerâmicos em diferentes condições clínicas. Além disso, a publicação 2 usou análises em MEV para explorar ainda mais o efeito de diferentes soluções de imersão na performance dos materiais à base de silicato de cálcio. É importante ressaltar que o emprego de diferentes

abordagens metodológicos é essencial para obtenção de resultados mais robustos, favorecendo análises integrativas dos dados obtidos[80].

Soluções alternativas como o uso de fluidos corporais simulados são empregadas para avaliar mais adequadamente a solubilidade e comportamento dimensional de materiais hidrofílicos[63,64]. Desta forma, a avaliação da solubilidade do Clinquer foi realizada empregando água destilada ou PBS. Foi observado maior ganho de massa em PBS em comparação a água destilada (Tabela 2, Publicação 1). Este resultado pode ser justificado pelo potencial de sorção de água do material e à interação dos íons de cálcio do clínquer com o fosfato do PBS[63,82], promovendo a formação de uma camada superficial de hidroxiapatita, o que poderia levar ao aumento de massa do material. Na publicação 2 e 3, o ácido butírico foi empregado para simular um ambiente ácido e PBS para mimetizar uma condição neutra. Além disso, o uso de tubos de dentina bovina, bem como o emprego de dentes humanos extraídos são importantes para avaliação do comportamento de cimentos biocerâmicos, considerando o imprescindível papel do substrato dentinário para o adequado desempenho desses materiais[83].

Materiais confeccionados a partir do Clinquer Angelus apresentam propriedades físicas adequadas para aplicabilidade clínica, especialmente quando manipulado com líquido com aditivos. O conhecimento do comportamento dos materiais em um ambiente ácido proporciona informações valiosas auxiliando ao clínico na escolha adequada do material endodôntico frente a lesões periradiculares. Estudos *in-vivo* devem ser encorajados para avaliar o comportamento de materiais à base de silicato de cálcio em diferentes condições clínicas.

5 CONCLUSÃO

De acordo com as publicações do presente estudo, pode-se concluir que:

Publicação 1: Clínquer <2 µm manipulado com líquido com aditivos proporciona menor tempo de presa final. Ambos tamanhos de partícula influenciam positivamente na capacidade de alcalinização do Clínquer, principalmente nos períodos iniciais. A adição do tungstato de cálcio e manipulação com líquido com aditivos apresentaram adequadas propriedades físicas para o Clínquer.

Publicação 2: Ambiente ácido influencia negativamente a qualidade de selamento na interface material/dentina, porosidade, e maior perda volumétrica dos materiais biocerâmicos. A manipulação do Clínquer com água destilada aumenta a porosidade, falhas na interface e promove maior perda de volume.

Publicação 3: Meio ácido prejudica as propriedades do Bio-C Sealer e AH Plus Jet, principalmente após imersão por 30 dias. A maior espessura de cimento no terço cervical prejudica a qualidade da obturação.

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APÊNDICE A – METODOLOGIA EXPANDIDA

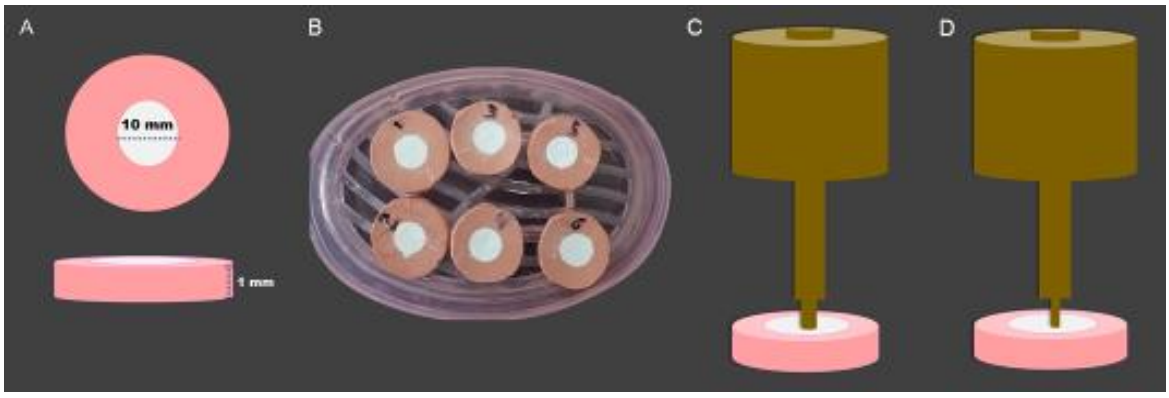
PUBLICAÇÃO 1

Avaliação das propriedades físico-químicas

Tempo de presa

O tempo de presa foi avaliado de acordo com as normas ISO 6876:2012[1] e norma ASTM C266-03[2] (Figura 1). Modelos de gesso tipo IV (Yamay, Atibaia, São Paulo, Brasil) foram confeccionados com 10 mm de diâmetro interno e 1 mm de altura (n=6), e mantidos em água destilada por 24h. Após manipulação, os materiais foram inseridos nos modelos de gesso e armazenados em estufa a 37°C e 95% de umidade relativa. Agulha de Gilmore de 100 ± 0,5g e 2 ± 0,1 mm de diâmetro foi empregada para determinação do tempo de presa inicial, conforme estabelecido pela norma ISO 6876. Posteriormente, uma agulha de Gilmore de 456 ± 0,5 g e 1,0 ± 0,1 mm de diâmetro foi utilizada para determinar o tempo de presa final, conforme estabelecido pela norma ASTM C266-03. O tempo de presa inicial e final foram considerados em minutos, desde a manipulação dos materiais até o momento em que as respectivas agulhas não pudessem produzir marcas na superfície dos cimentos.

Figura 1 - Imagem representativa do tempo de presa dos materiais experimentais



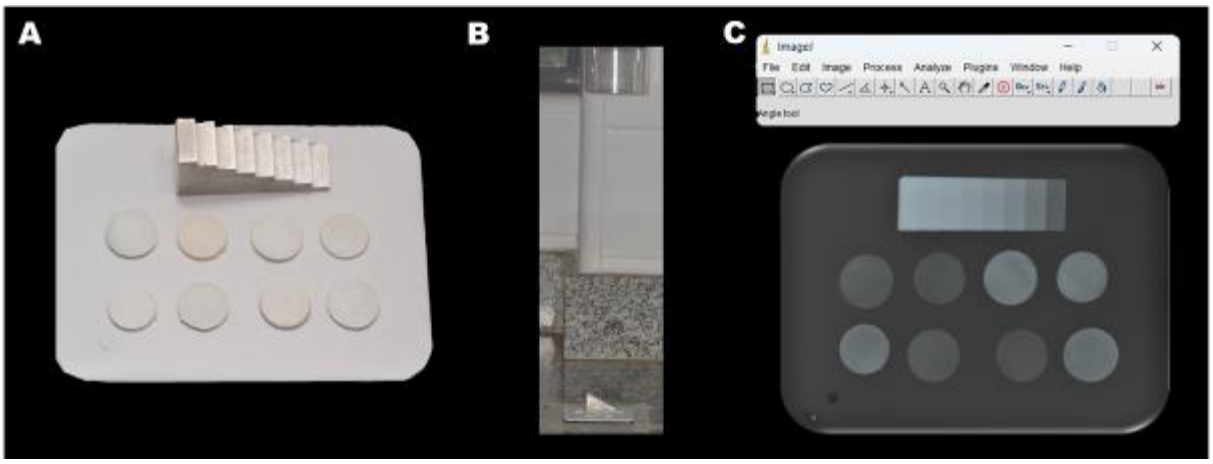
A, Corpos de prova (n=6) em modelos de gesso de 10 mm de diâmetro e 1 mm de altura. B, Materiais experimentais inseridos nos modelos de gesso e mantidos em estufa a 37°C. C, Medição com agulha Gilmore (100 ± 0,5 g e 2,0 ± 0,1 mm de diâmetro) para determinar tempo de presa inicial. D, Medição com agulha Gilmore (456 ± 0,5 g e 1,0 ± 0,1 mm de diâmetro) para determinar tempo de presa final.

Fonte: Arquivo pessoal da autora.

Radiopacidade

Após manipulação, os cimentos foram inseridos em moldes circulares plásticos com 10 mm de diâmetro interno e 1 mm de altura (n=6). Os espécimes foram mantidos em estufa a 37°C, 95% de umidade, 24 horas para garantir presa. Após isso, o disco de cada material e uma escala de alumínio foi colocado sobre um filme oclusal (Insight – Kodak Comp., Rochester, NY) e tomada radiográfica (Aparelho de raios-X GE 1000 - General Eletric, Milwaukee, WI) foi realizada por meio dos seguintes parâmetros: 60 kV, 7 mA, 0,32 pulsos por segundo e distância foco-filme de 33 cm. Os filmes foram processados, digitalizados e avaliados empregando o programa Imagem J (National Institutes of Health, Bethesda, USA). O processo metodológico do teste de radiopacidade seguindo as diretrizes ISO 6876:2012 pode ser visualizado na Figura 2.

Figura 2 - Imagem representativa da radiopacidade dos materiais



A, Corpos de prova com 10 mm de diâmetro e 1 mm de altura. B, Tomada radiográfica empregando os parâmetros definidos. C, Imagem digitalizada e avaliada pelo programa Imagem J.

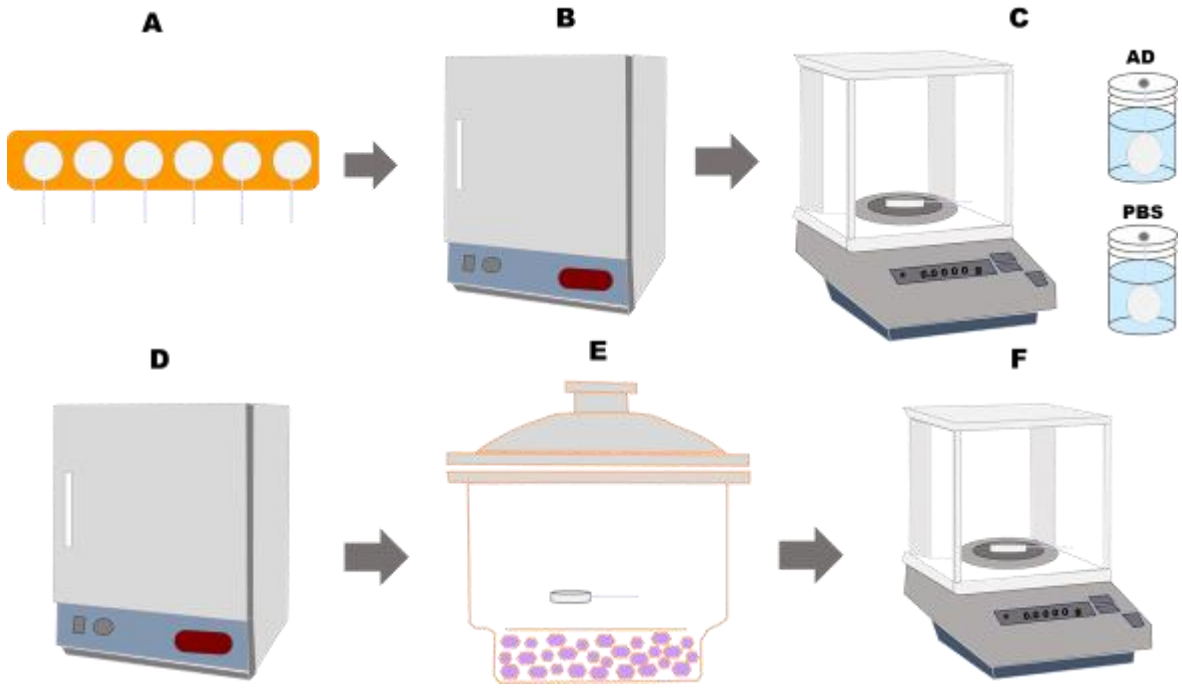
Fonte: Arquivo pessoal da autora.

Solubilidade

Moldes circulares plásticos foram confeccionados com 1,5 mm de altura e 7,75 mm de diâmetro interno[3] e preenchidos com cada material (n=6), inserindo um fio de nylon incluído na mistura de cada cimento. O conjunto foi armazenado em estufa a 37° C por 24h. Os corpos de prova foram removidos dos moldes, pesados em balança de precisão HM-200 (A & D Enginnering, Inc., Bradford, MA, EUA) para determinação da massa inicial. Posteriormente, as amostras foram suspensas por meio dos fios de

nylon no interior de recipientes plásticos com tampa contendo 7,5 mL de água destilada e deionizada ou solução salina tamponada com fosfato (PBS). Os recipientes permaneceram em estufa a 37°C por 7 dias. Após esse período, os corpos-de-prova foram removidos das soluções, enxaguados e colocados em dessecador com sílica. As amostras foram pesadas a cada 24 horas até a estabilização da massa final (0,001g). O percentual de perda de massa foi determinado levando em consideração a diferença entre a massa inicial e final antes e após imersão em água destilada ou PBS após 7 dias. O teste de solubilidade está representado na Figura 3.

Figura 3 – Figura esquemática do teste de solubilidade



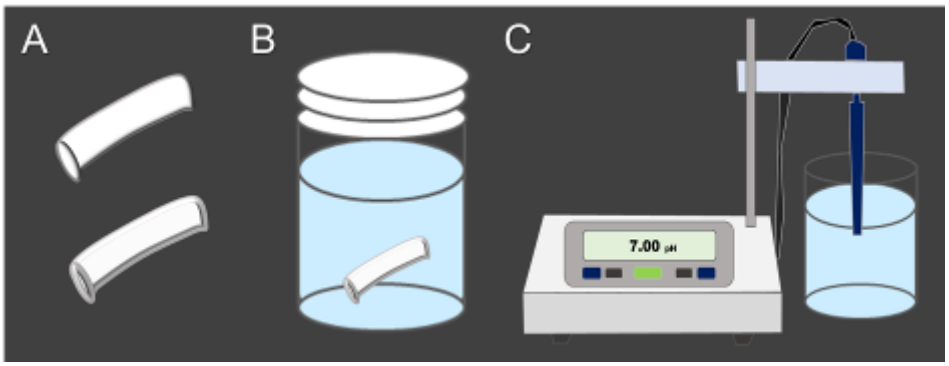
A, Moldes plásticos circulares com 1,5 mm de altura e 7,75 mm de diâmetro interno preenchidos com os materiais (n=12). B, Armazenamento das amostras em estufa a 37° C por 24h. C, Determinação da massa inicial através de balança de precisão e imersão em água destilada (AD) ou PBS por 7 dias (n=6). D, Acondicionamento das amostras em estufa a 37°C. E, Corpos de prova armazenados em dessecador com sílica. F, Determinação da massa final das amostras por meio de balança de precisão.

Fonte: Arquivo pessoal da autora.

pH

Tubos de polietileno (10 mm de comprimento e 1,6 mm de diâmetro) foram preenchidos com cada material (n=10). Cada tubo foi imerso em 10 mL de água destilada em um pote com tampa e mantido em estufa a 37°C. Após cada período experimental, os tubos foram colocados em novos frascos com 10 mL de água destilada e deionizada. Os períodos experimentais foram 1, 7, 14, 21 e 28 dias. O pH das soluções foi medido por meio de um pHmetro digital (Mettler Toledo, Columbus, Ohio, EUA) previamente calibrado. Frascos contendo apenas água destilada e deionizada foram utilizados como grupo controle. O teste de pH empregado no presente estudo está representado na Figura 4.

Figura 4 - Imagem representativa do teste de pH



A, Tubos de polietileno com 10 mm de comprimento e 1,6 mm de diâmetro. B, Tubos imersos em frascos contendo 10 mL de água destilada e deionizada. C, pH foi mensurado após 1, 7, 14, 21 e 28 dias utilizando o pHmetro digital.

Fonte: Arquivo pessoal da autora.

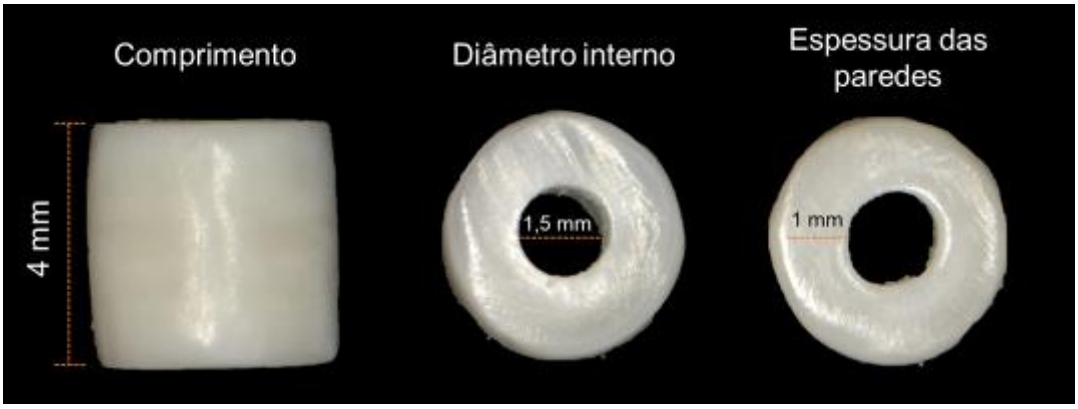
Publicação 2

Preparo dos espécimes

Dentes bovinos extraídos foram empregados (n=60). Exames radiográficos (Kodak RVG 6100 Digital Radiography System, Marne-la-Vallée, França) foram realizados para confirmar ausência de anomalias. As raízes foram seccionadas transversalmente em máquina corte Isomet 1000 (Buehler Ltda, Lake Bluff, IL, EUA), obtendo espécimes com 4 mm de comprimento. Posteriormente, um único operador previamente treinado e calibrado empregou um delineador (Bio-Art, São Carlos, São

Paulo, Brasil) para a fixação de cada amostra e broca Gates-Glidden #6 (Dentsply Maillefer, Ballaigues, Suíça) acoplada ao motor e baixa rotação (Micromotor N270 e Contra-ângulo; Dabi-Atlante, Ribeirão Preto, São Paulo, Brasil) para o preparo do canal radicular[4]. Para obtenção da espessura das paredes foi utilizado a broca cilíndrica (Maxicut 1503; American Burrs, Palhoça, Santa Catarina, Brasil) e um paquímetro digital (Mitutoyo Corporation; São Paulo, SP, Brasil) para confirmação do grossor. Cada amostra foi confeccionada com os seguintes parâmetros: 4 mm de comprimento, 1,5 mm de diâmetro interno e 1 mm aproximadamente de espessura (Figura 5). Durante todo preparo, os canais foram inundados com solução de hipoclorito de sódio 1% (NaOCl) utilizando seringa Ultradent (South Jordan, UT, EUA) com agulha Navitip 30G (Ultradent). A irrigação final foi realizada com 5 mL de NaOCl 2,5% e 5 mL de EDTA 17%, por 3 minutos, seguido de 5 mL de água destilada. Posteriormente, as amostras foram radiografadas para confirmação da espessura das paredes e foram estocados em solução de timol 0,1% a 5°C até o momento do uso.

Figura 5 - Parâmetros de confecção dos tubos de dentina bovina



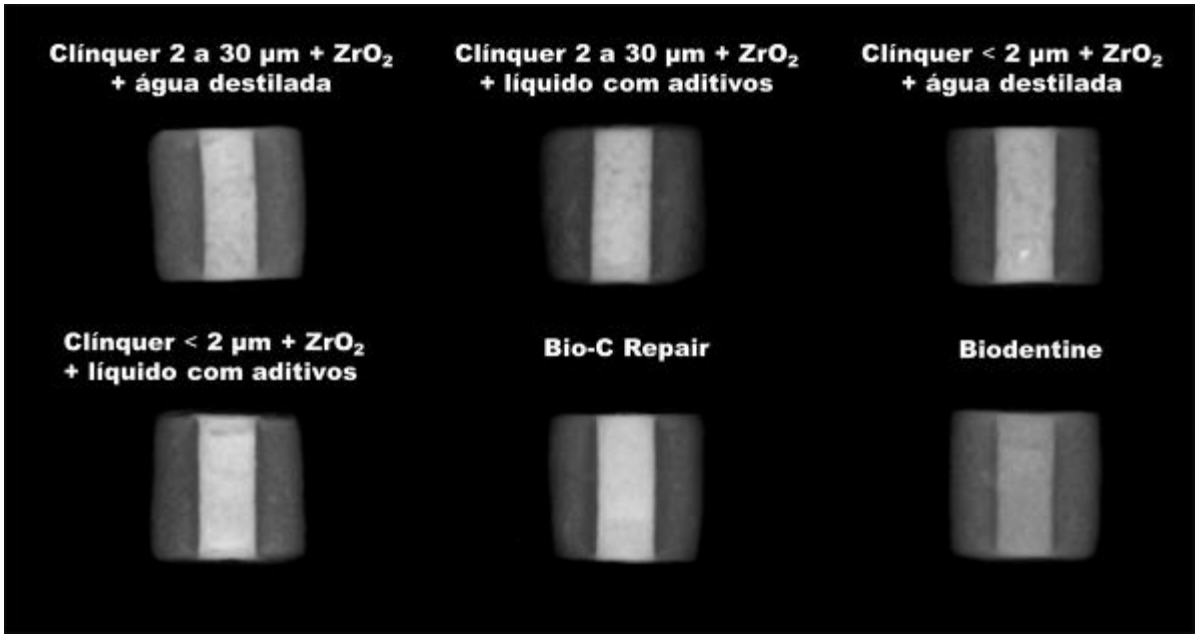
Fonte: Arquivo pessoal da autora.

Preenchimento dos espécimes

Após preparo, os tubos de dentina foram imersos em água destilada e armazenados em estufa a 37°C por 24 horas. Em seguida, as cavidades foram preenchidas com Clínquer 2 a 30 µm + ZrO_2 + água destilada, Clínquer 2 a 30 µm + ZrO_2 + líquido, Clínquer < 2 µm + ZrO_2 + água destilada, Clínquer < 2 µm + ZrO_2 + líquido, Bio-C Repair ou Biodentine (n=10) utilizando um kit de condensadores (Ref.: 324501, nº 2, 3 e 4; Golgran; São Caetano do Sul, SP, Brasil). As amostras foram

radiografadas para confirmação do adequado preenchimento (Figura 6) e foram armazenadas em estufa a 37°C e umidade relativa de 95% por 24 horas.

Figura 6 – Radiografias comprobatórias de cada grupo experimental para verificação do adequado preenchimento dos espécimes

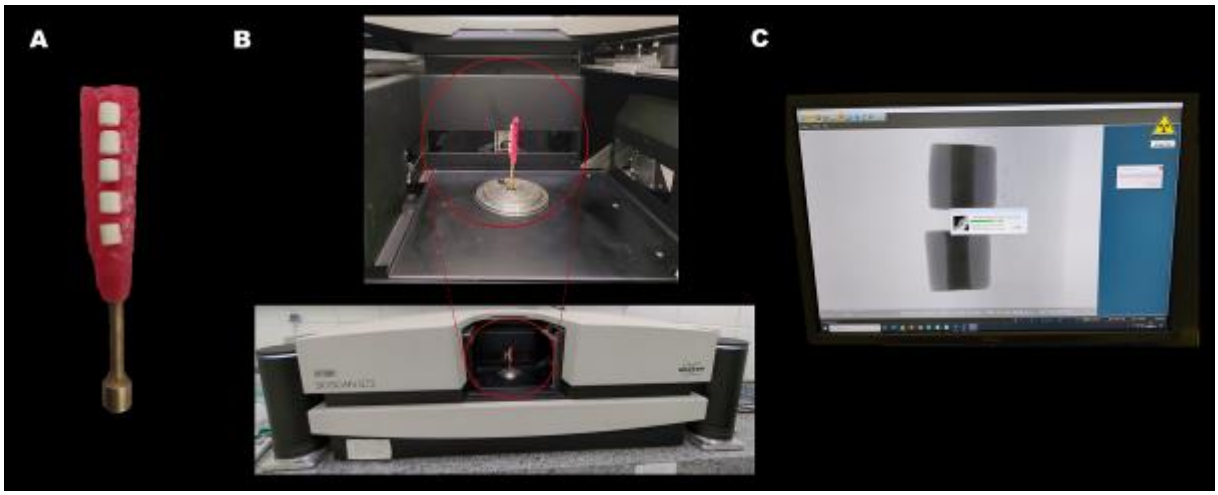


Fonte: Arquivo pessoal da autora.

Escaneamento em micro-CT e imersão dos espécimes

Os espécimes foram submetidos a escaneamento inicial em micro-CT (SkyScan 1272, Bruker, Kontich, Bélgica) utilizando os seguintes parâmetros: tamanho de voxel 8,74 µm, passo de rotação de 0,5, frame 3, filtro Al 1 mm, rotação 180°, 80 kV e 125 uA. Em seguida, cada espécime foi imerso em 1.5 mL de ácido butírico (Sigma Aldrich, Barueri, SP, Brasil, pH: 4,1) ou PBS (Sigma, pH: 7,0) (n= 5) e armazenados em estufa a 37 ºC por 7 e 28 dias. A solução de ácido butírico foi trocada a cada 24 horas para garantir a estabilidade do pH. Escaneamentos em micro-CT após períodos de 7 e 28 dias com os mesmos parâmetros descritos anteriormente foram conduzidos. O passo-a-passo para realizar os escaneamentos das amostras em micro-CT pode ser visualizado na Figura 7.

Figura 7 - Imagem representativa do escaneamento das amostras em diferentes períodos



A, Posicionamento das amostras em cera e mandril para garantir estabilidade durante os escaneamentos. B, Fixação do mandril em posição vertical no aparelho de micro-CT. C, Escaneamento das amostras após determinação de parâmetros específicos.

Fonte: Arquivo pessoal da autora.

Análise em micro-CT

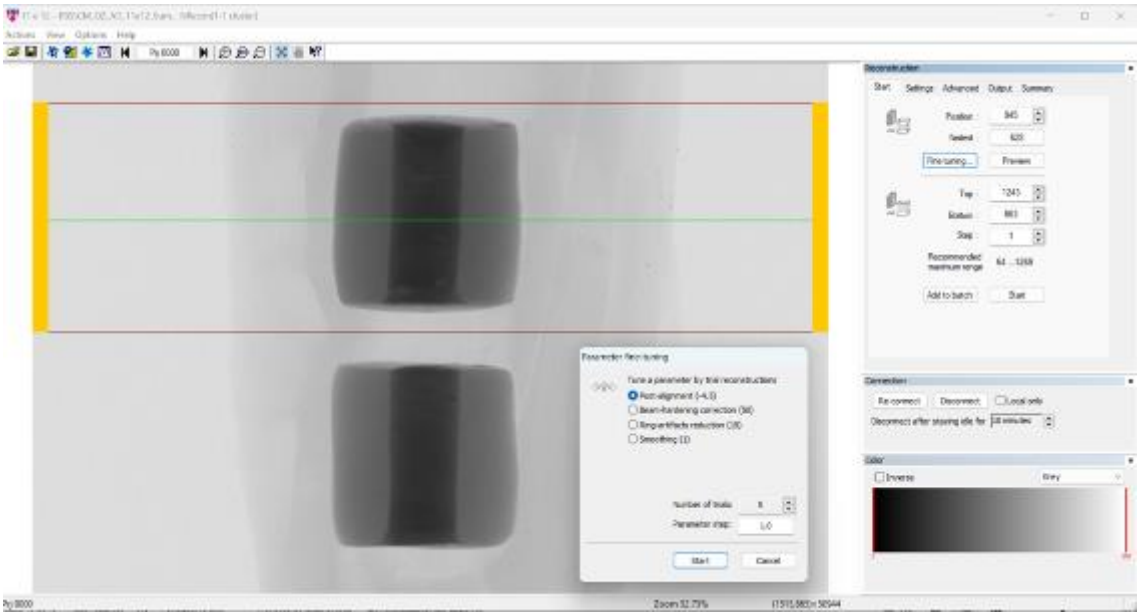
As reconstruções das imagens nos diferentes períodos experimentais foram realizadas no software NRecon (V.1.6.3; SkyScan, Bélgica) após definição de parâmetros individuais para cada material (Quadro 1 e Figura 8). As imagens reconstruídas em 3D foram registradas nos diferentes períodos utilizando o software Data Viewer (v.1.5.1; SkyScan, Bélgica) (Figura 9).

Quadro 1 - Definição dos parâmetros beam-hardening correction, ring-artifacts reduction e smoothing para cada material nos diferentes períodos de avaliação

Após imersão em Ácido Butírico ou PBS			
	Inicial	**7 dias**	**28 dias**
Clínquer 2 a 30 µm + ZrO_2 + água destilada	Beam-hardening: 50, Ring: 19, Smoothing: 1	Beam-hardening: 50, Ring:19, Smoothing: 1	Beam-hardening: 50, Ring:19, Smoothing: 1
Clínquer 2 a 30 µm + ZrO_2 + líquido com aditivos	Beam-hardening: 50, Ring: 19, Smoothing: 1	Beam-hardening: 50, Ring: 19, Smoothing: 1	Beam-hardening: 50, Ring: 19, Smoothing: 1
Clínquer < 2 µm + ZrO_2 + água destilada	Beam-hardening: 60, Ring: 19, Smoothing: 1	Beam-hardening: 60, Ring: 19, Smoothing: 1	Beam-hardening: 60, Ring: 19, Smoothing: 1
Clínquer < 2 µm + ZrO_2 + líquido com aditivos	Beam-hardening: 60, Ring: 19, Smoothing: 1	Beam-hardening: 60, Ring: 19, Smoothing: 1	Beam-hardening: 60, Ring: 19, Smoothing: 1
Bio-C Repair	Beam-hardening: 48, Ring: 19, Smoothing: 1	Beam-hardening: 50, Ring: 19, Smoothing: 1	Beam-hardening: 50, Ring: 19, Smoothing: 1
Biodentine	Beam-hardening: 70, Ring: 19, Smoothing: 1	Beam-hardening: 72, Ring: 19, Smoothing: 1	Beam-hardening: 70, Ring: 19, Smoothing: 1

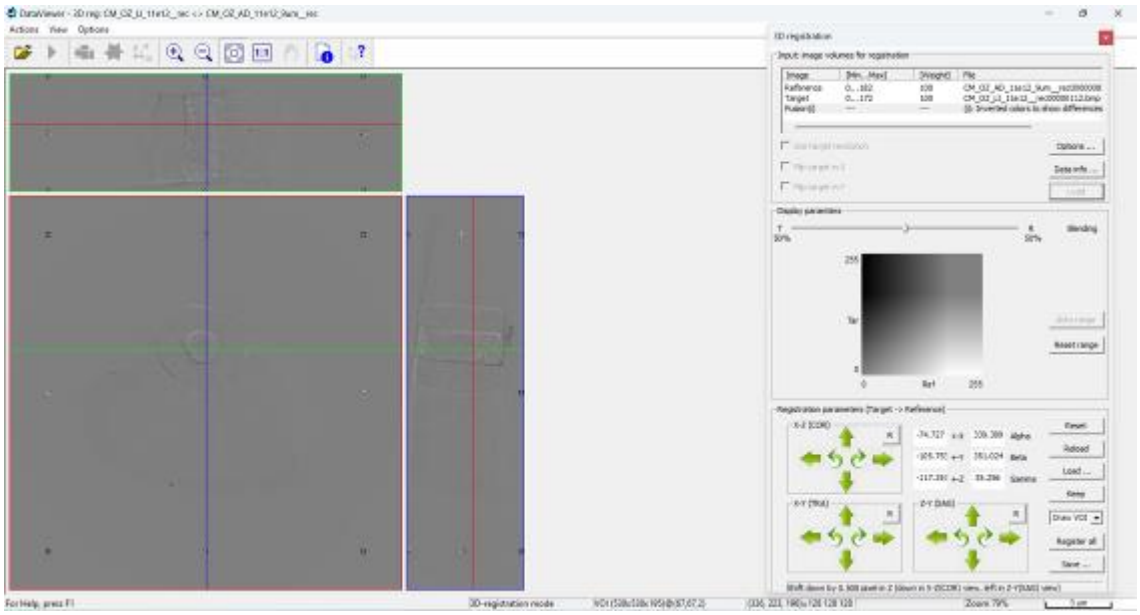
Fonte: Elaboração própria.

Figura 8 - Imagem representativa da reconstrução das imagens através de parâmetros específicos usando software NRecon



Fonte: Arquivo pessoal da autora.

Figura 9 - Imagem representativa da sobreposição das imagens nos diferentes períodos experimentais no Software DataViewer

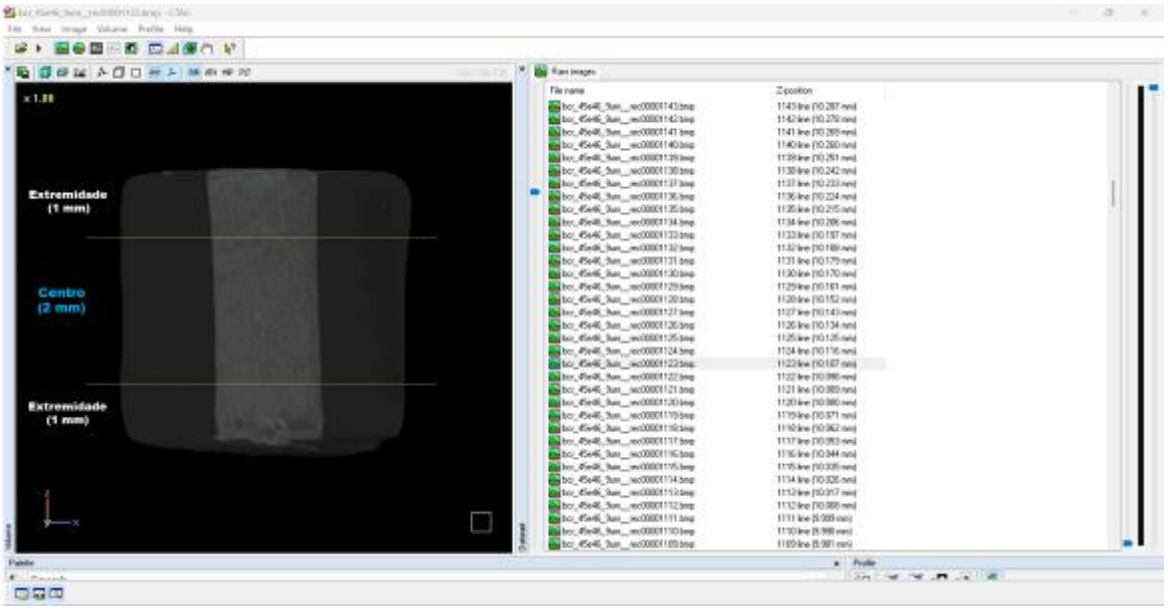


Fonte: Arquivo pessoal da autora.

As imagens registradas após imersão nas soluções por 7 e 28 dias foram analisadas quantitativamente no software CTAn (V.1.15.4.0; SkyScan, Bélgica). Para cada análise, o espécime foi divido em três partes: 1 mm para a extremidade superior,

1 mm para a extremidade inferior (extremidades) e 2 mm para o centro da amostra (parte interna), conforme ilustrado na Figura 10.

Figura 10 - Imagem representativa da divisão da amostra para análises das variáveis



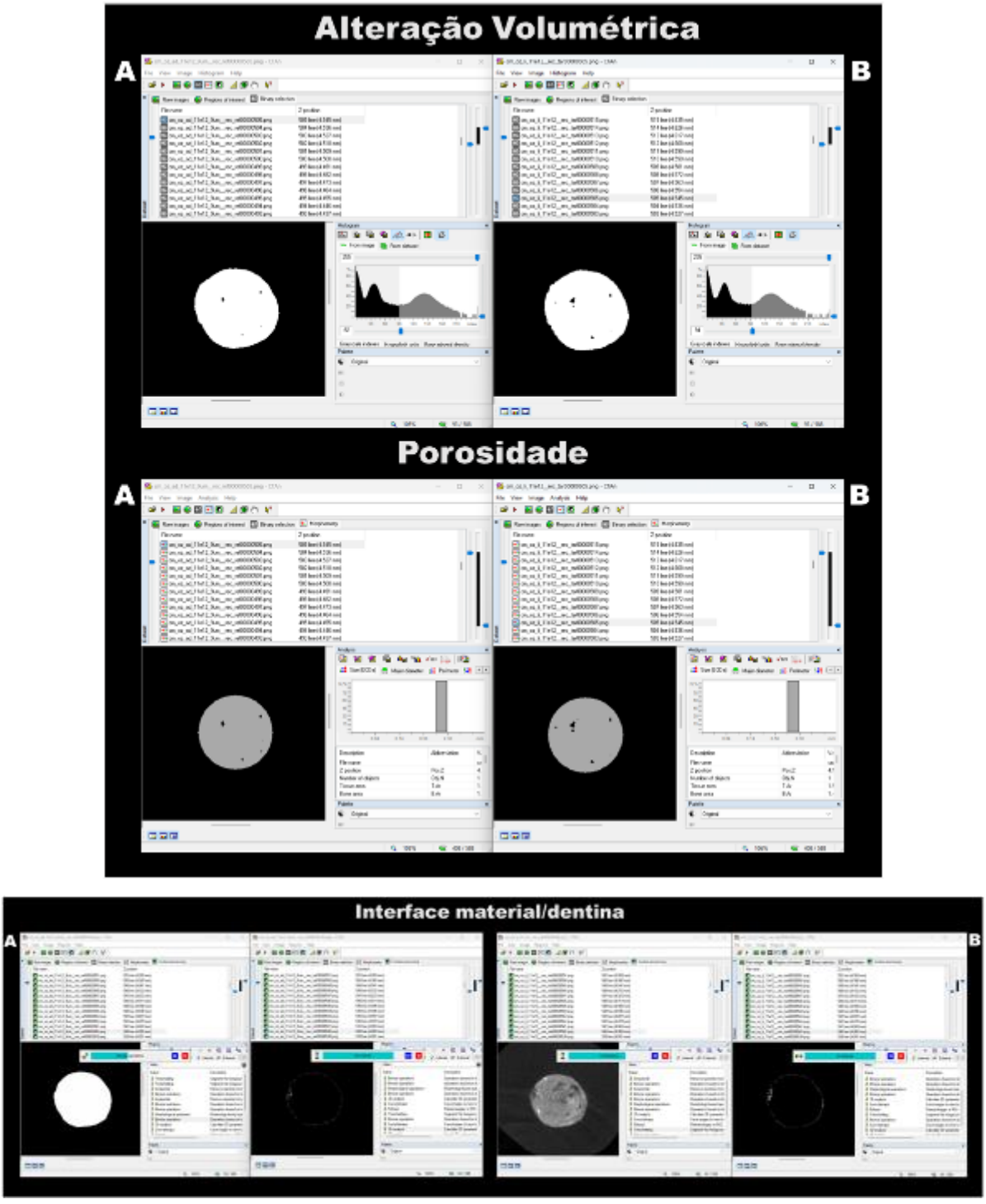
Fonte: Arquivo pessoal da autora.

A alteração volumétrica foi calculada determinando o volume inicial (mm^3) e após imersão nos diferentes meios após 7 e 28 dias. A porosidade foi avaliada com base nos valores obtidos após presa dos materiais e depois da imersão em ácido butírico ou PBS após ambos períodos experimentais.

O percentual de falhas da interface material/dentina foi realizado com base na metodologia descrita por Gandolfi et al.[5] (2013). A distribuição 3D das falhas na interface em um volume de interesse pré-definido (VOI) foi calculada após o preenchimento e após a imersão em PBS ou ácido butírico por 7 e 28 dias. Após definição do *bottom* e *top* das extremidades ou centro da amostra, thresholding adaptativo foi utilizado para reconhecer cada objeto de interesse. Cada 3D-VOI foi definido como o volume da interface, englobando parte da dentina e parte do material de preenchimento por meio de um "*task list"* específicos com operações aritméticas e lógicas entre as seções sobrepostas. Assim, falhas com tamanhos a partir de 8,74 µm dentro do VOI foram detectados. Um esquema representativo das análises pode ser

visto na Figura 11. Imagens representativas foram elaboradas empregando o software CTVOx (v.3.2; Bruker-microCT).

Figura 11 - Imagem representativa das análises da interface material/dentina, alteração volumétrica e porosidade das amostras

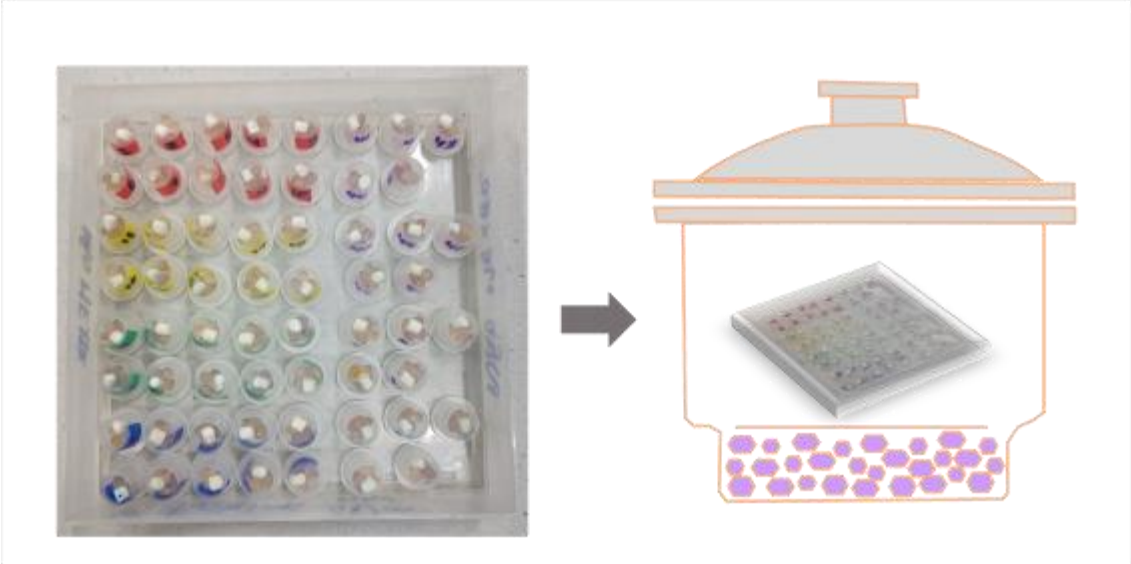


A, Reference e B, Target nos diferentes períodos experimentais.

Fonte: Arquivo pessoal da autora.

Após o escaneamento das amostras, cada tubo de dentina foi devidamente identificado e armazenado no dessecador com sílica para posterior análises em MEV (Figura 12).

Figura 12 – Imagem representativa da identificação de cada amostra colocada individualmente na sílica e armazenada no dessecador para posterior análise em MEV



Fonte: Arquivo pessoal da autora.

Análises da superfície em MEV

Após imersão em ácido butírico ou PBS (n=3 de cada grupo) por 28 dias, as amostras foram secas com papel absorvente e mantidos em um dessecador a vácuo contendo sílica por 24h. Os tubos de dentina com materiais foram revestidos com carbono e visualizados em MEV (JEOL JSM-6610LV, Tóquio, Japão) em três ampliações (27x, 100x e 500x) em modo de elétrons retroespalhado secundário. Todas as análises foram realizadas a 12 kV e SS 40. As superfícies de cada amostras foram analisadas.

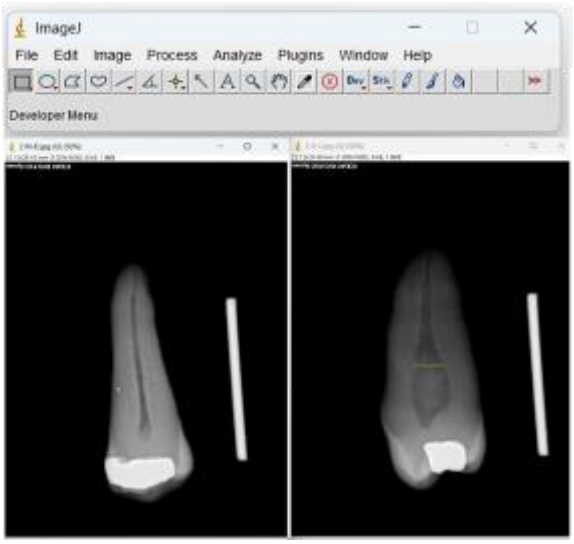
Publicação 3

Seleção dos dentes

Após aprovação pelo Comitê de Ética em Pesquisa (CAAE: 41926620.6.0000.5416), foram selecionados 32 dentes humanos extraídos com canais radiculares ovais por análise radiográfica digital (Kodak RVG 6100; Digital Radiography System, Marne-la-

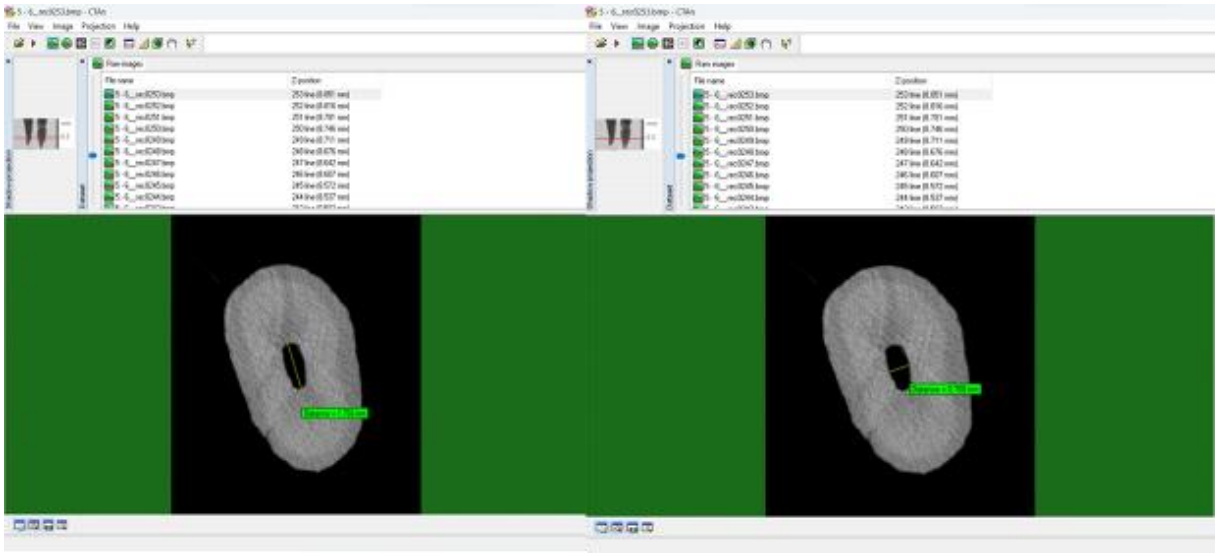
Vallée, France). As radiografias foram realizadas no sentido vestíbulo-lingual e mésio-distal das raízes e as imagens foram analisadas através do *software* Image J (National Institutes of Health, Bethesda, USA) (Figura 13). Posteriormente, os espécimes considerados ovais radiograficamente, foram submetidos a escaneamento para seleção, por meio de micro-CT (SkyScan 1176, Bruker-microCT, Kontich, Bélgica) utilizando os seguintes parâmetros: 80 kV, 300 uA, 180° rotação, passo de rotação 0.5, média de frame 3, filtro de cobre e alumínio, tempo de exposição de 125 ms e tamanho de voxel de 35 µm. A relação de diâmetro (RD) foi obtida mensurando o comprimento vestíbulo-lingual e mésio-distal do canal. Os canais radiculares foram considerados ovais quando diâmetro vestíbulo-lingual era igual ou maior que 2 vezes o diâmetro mesio-distal[6] a 9 mm do ápice radiográfico[7], através do *software* CTAn (v.1.14.4, SkyScan, Bélgica) (Figura 14).

Figura 13 – Imagem representativa das amostras no *software* ImageJ no sentido vestíbulo-lingual e mésio-distal para determinação dos canais radiculares ovais



Fonte: Arquivo pessoal da autora.

Figura 14 – Imagem representativa das amostras no *software* CTAn no sentido vestíbulo-lingual e mésio-distal para determinação do canal radicular oval



Fonte: Arquivo pessoal da autora.

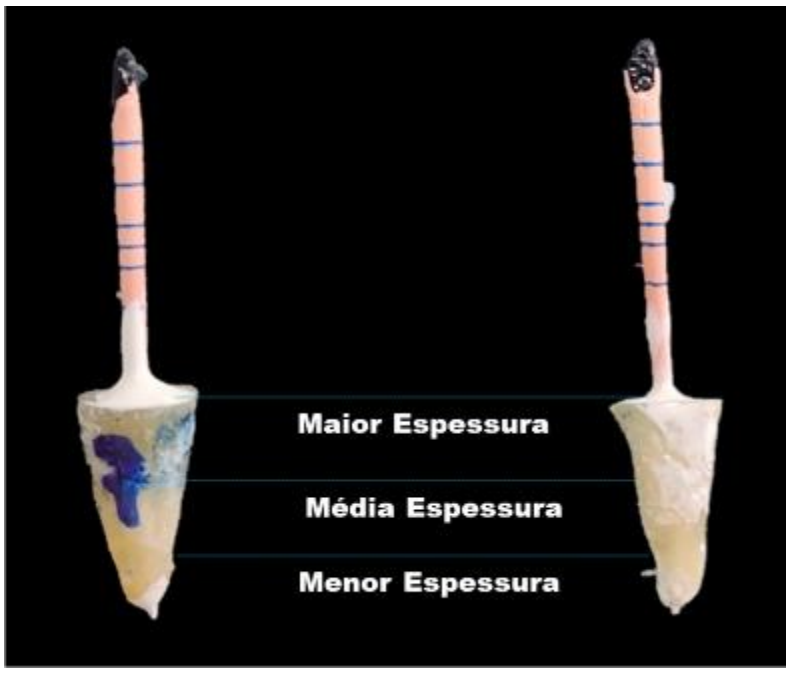
Preparo e obturação dos canais radiculares

Após o acesso coronário, os canais foram explorados com lima K 15 e 20 (Dentsply, Maillefer, Ballaigues, Suíça). O comprimento de trabalho (CT) foi determinado 1 mm além do forame apical. As raízes foram moldadas com silicone de condensação (Oranwash, Zhermack SpA, Badia Polesine, Itália) para simular o ligamento periodontal e estabilizar as amostras durante os procedimentos. Posteriormente, um único operador previamente treinado e calibrado preparou os canais com ProDesign Logic (Easy Equipamentos Odontológicos, Belo Horizonte, MG, Brasil) 40/.05 a 950 RPM e 4Ncm acionado em motor elétrico VDW SILVER (VDW GmbH, Munique, Alemanha). Durante todo preparo, os canais foram irrigados com 5 mL de hipoclorito de sódio a 1% (NaOCl; Ciclo Farma, Serrana, SP, Brazil) utilizando uma seringa de 5 mL (Ultradent, South Jordan, UT) com agulha Navitip 30G (Ultradent). Após preparo, foi realizada secção dos 10 milímetros apicais das raízes em máquina de corte Isomet 1000 (Buehler Ltd, Lake Bluff, Illinois, United States). Posteriormente, a irrigação final dos espécimes foi realizada com 5 mL de NaOCl 2,5% e 5 mL de EDTA 17% (Biodinâmica, Ibiporã, PR, Brasil) por 3 minutos, seguido por 5 mL de água destilada.

Após 24h de imersão em água destilada, os canais foram aspirados e o excesso de umidade foi removido por meio de cones de papel absorvente 40/.05 (EndoTanari,

Endomil Distribuidora LTDA, Santa Luzia, MG). Os canais foram obturados pela técnica do cone único, utilizando cones 40/.04 (Tanariman, Manacapuru, AM, Brasil) transpassando o forame apical até ajuste, e divididos em dois grupos: Bio-C Sealer ou AH Plus Jet (n=16), proporcionando menor espessura no terço apical (ME), média espessura no terço médio (MDE) e maior espessura no terço cervical (MA) (Figura 15).

Figura 15 - Imagem representativa da obturação das amostras ilustrando a espessura de cimento estabelecida de acordo com cada terço radicular

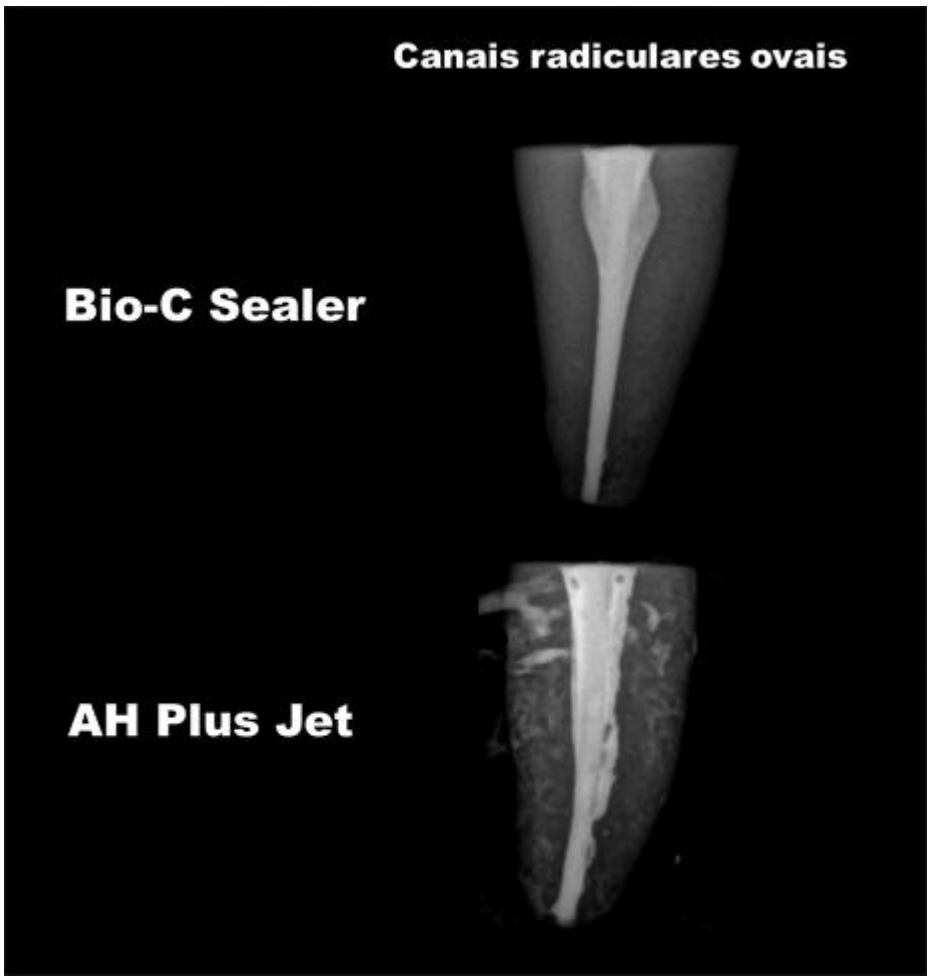


Fonte: Arquivo pessoal da autora.

Bio-C Sealer foi injetado no canal radicular cerca de 4 mm aquém do comprimento de trabalho, usando seringas e agulhas plásticas fornecidas pelo fabricante. AH Plus Jet foi manipulado seguindo as instruções do fabricante e levado ao canal com auxílio de instrumento tipo K40 manual e lentulo #40 (Dentsply Sirona, Ballaigues, Suiça) em baixa rotação no sentido horário. Os cones principais de guta-percha foram levados ao canal após introdução do cimento envoltos por cada material. Os excessos de guta-percha foram cortados e compactados com calcadores Paiva previamente aquecidos. Radiografias digitais em sentido vestíbulo-lingual e mesio-distal foram realizadas para avaliar a qualidade do preenchimento do canal radicular

(Figura 16). As amostras foram acondicionadas em estufa a 37º C e 95% de umidade por 7 dias.

Figura 16 - Radiografias comprobatórias da qualidade de preenchimento pelos diferentes cimentos em canais radiculares ovais



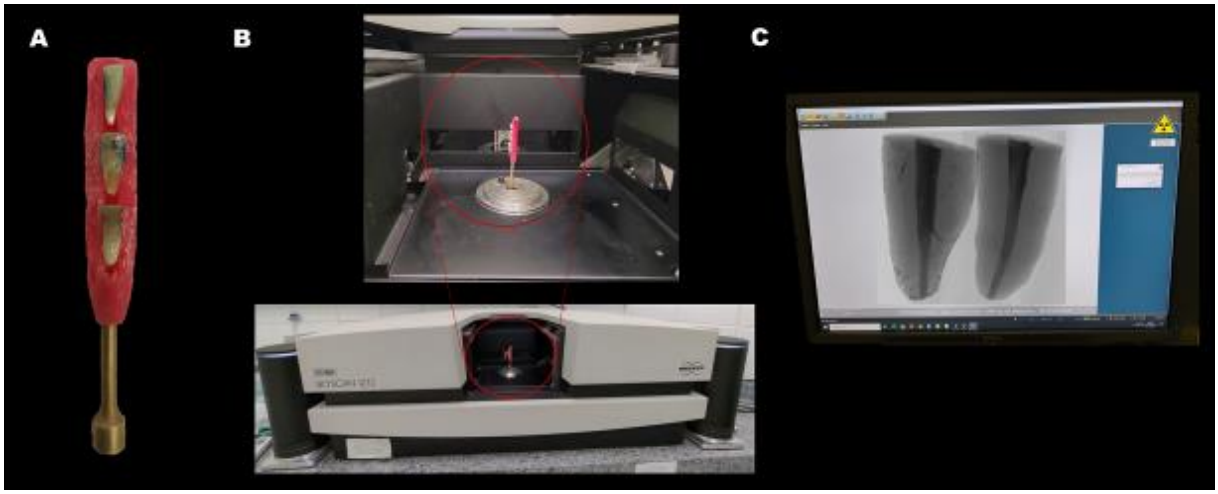
Fonte: Arquivo pessoal da autora.

Escaneamento em micro-CT e imersão dos espécimes

Os espécimes foram submetidos a escaneamento inicial em micro-CT (SkyScan 1272, Bruker, Kontich, Bélgica) utilizando os seguintes parâmetros: tamanho de voxel 8,74 µm, passo de rotação de 0,5, frame 3, filtro Al 0.5 + Cu .038, rotação 180°, 90 kV e 110 uA. Em seguida, cada espécime foi imerso em 1,5 mL de PBS (Sigma, pH: 7,0) ou ácido butírico (Sigma, pH: 4,1) (n= 8) e armazenados em estufa a 37 ºC por 7 e 30 dias. A solução de ácido butírico foi trocada a cada 24 horas para garantir a estabilidade do pH. Novos escaneamentos em micro-CT foram conduzidos após os diferentes períodos experimentais usando os mesmos

parâmetros descritos no escaneamento inicial. O passo-a-passo para realizar os escaneamentos das amostras em micro-CT pode ser visualizado na Figura 17.

Figura 17 - Imagem representativa do escaneamento das amostras em diferentes períodos



A, Posicionamento das amostras em cera e mandril para garantir estabilidade durante os escaneamentos. B, Fixação do mandril em posição vertical no aparelho de micro-CT. C, Escaneamento das amostras após determinação de parâmetros específicos.

Fonte: Arquivo pessoal da autora.

Análise em micro-CT

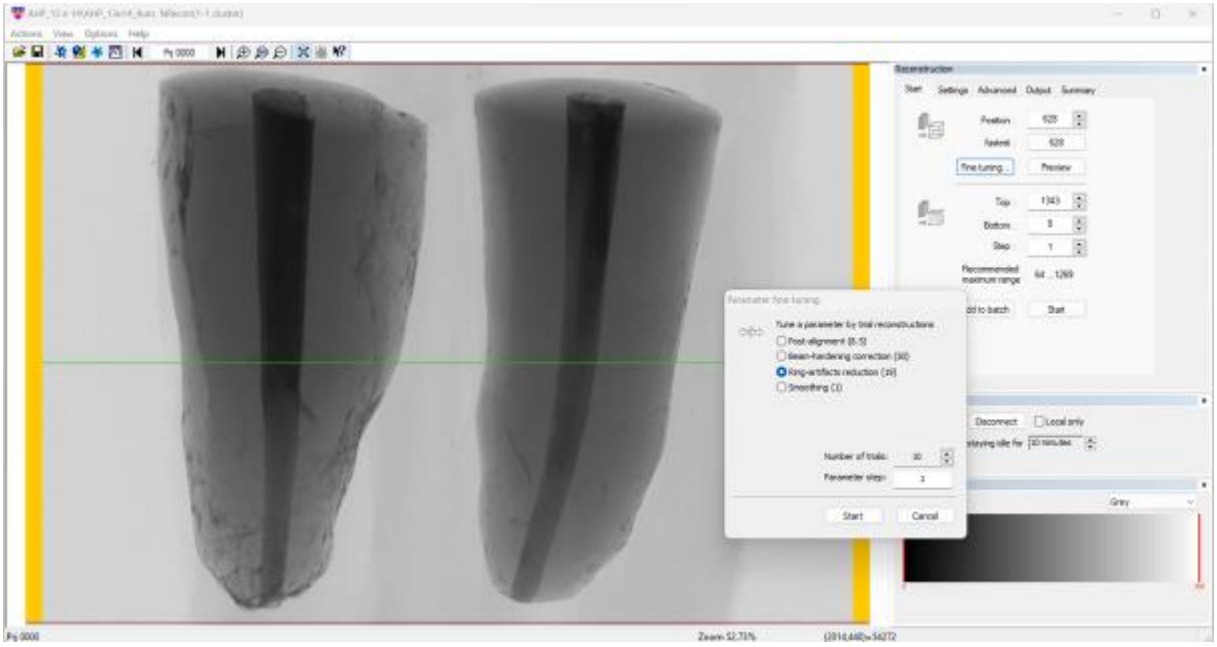
As reconstruções das imagens nos diferentes períodos experimentais foram realizadas no software NRecon (V1.6.3; SkyScan, Bélgica) após definição de parâmetros individuais para cada material (Quadro 2 e Figura 18). As imagens reconstruídas em 3D foram registradas nos diferentes períodos utilizando o software Data Viewer (V1.5.1; SkyScan, Bélgica) (Figura 19).

Quadro 2 - Definição dos parâmetros beam-hardening correction, ring-artifacts reduction e smoothing para cada material nos diferentes períodos de avaliação

Após imersão em PBS ou Ácido butírico			
	Inicial	**7 dias**	**30 dias**
Bio-C Sealer	Beam-hardening: 40, Ring: 19, Smoothing: 1	Beam-hardening: 60, Ring:19, Smoothing: 1	Beam-hardening: 40, Ring:19, Smoothing: 1
AH Plus Jet	Beam-hardening: 50, Ring: 19, Smoothing: 1	Beam-hardening: 50, Ring: 19, Smoothing: 1	Beam-hardening: 40, Ring: 19, Smoothing: 1

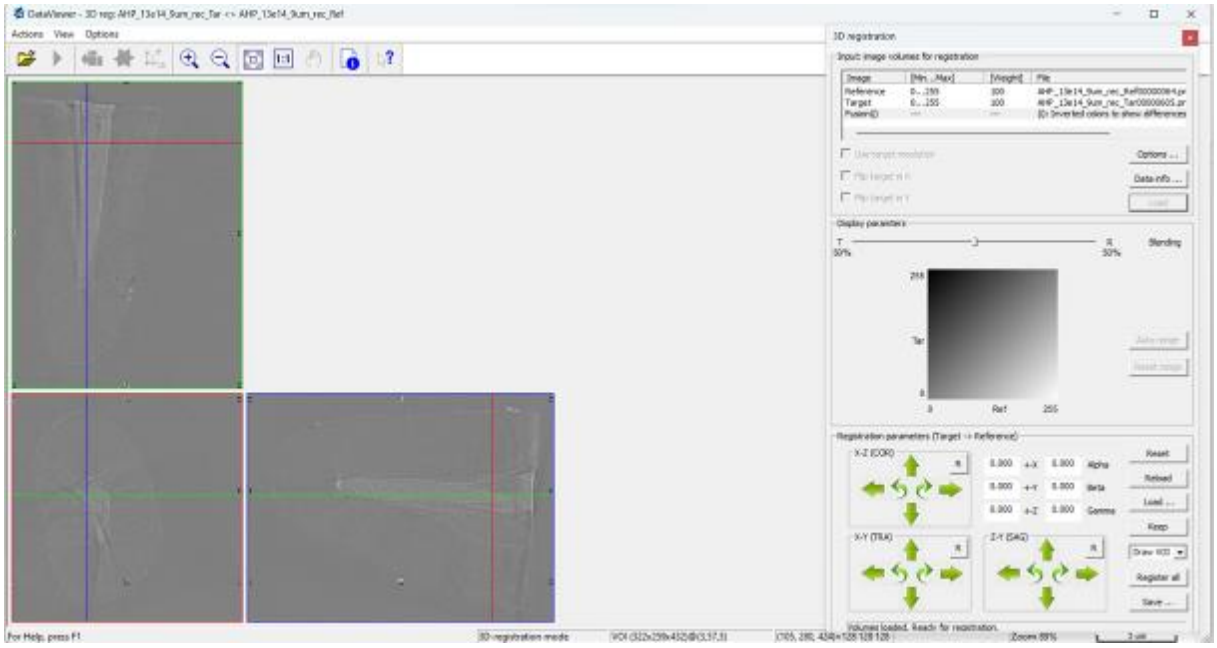
Fonte: Elaboração própria.

Figura 18 – Imagem representativa da reconstrução das imagens através de parâmetros específicos usando software NRecon



Fonte: Arquivo pessoal da autora.

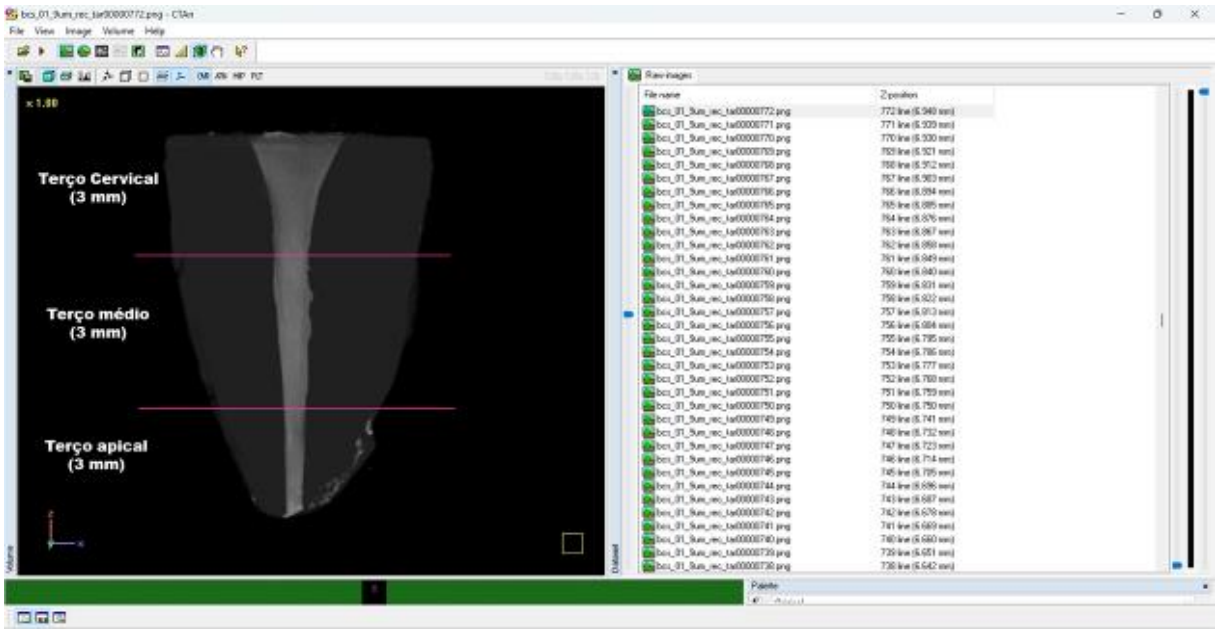
Figura 19 - Imagem representativa da sobreposição das imagens nos diferentes períodos experimentais no Software DataViewer



Fonte: Arquivo pessoal da autora.

As imagens registradas após imersão em PBS ou ácido butírico após 7 e 30 dias foram analisadas quantitativamente no software CTAn (V1.15.4.0; SkyScan, Bélgica). As avaliações foram realizadas para cada terço do espécime de acordo com o material obturador e espessura do cimento: menor espessura (ME, 3 mm da região apical), média espessura (MDE, 3 mm do terço médio) ou maior espessura (MA, 3 mm da região cervical) (Figura 20).

Figura 20- Imagem representativa da divisão da amostra para análises das variáveis



Fonte: Arquivo pessoal da autora.

A alteração volumétrica foi calculada entre o volume inicial e após a imersão por 7 e 30 dias. A porosidade após a presa e a influência da imersão em ácido butírico ou PBS nos dois períodos foi quantificada em porcentagem. O percentual de falhas da interface material/dentina foi realizado com base na metodologia descrita por Gandolfi et al. (2013)[5] . A distribuição 3D das falhas na interface em um volume de interesse pré-definido (VOI) foi calculada após o preenchimento e após a imersão em PBS ou ácido butírico por 7 e 30 dias. Após a definição do *bottom* e *top* de cada terço, o thresholding adaptativo foi utilizado para reconhecer cada objeto de interesse. Cada 3D-VOI foi definido como o volume de interface, englobando parte da dentina e parte do material de preenchimento por meio de um ¨*task list*¨ com operações aritméticas e lógicas entre as seções sobrepostas. Assim, falhas com tamanhos a partir de 8,74 µm dentro do VOI foram detectados. Imagens representativas foram elaboradas empregando o software CTVOx (v.3.2; Bruker-microCT).

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ANEXO A – CERTIFICADO DO COMITÊ DE ÉTICA

Projeto de Pesquisa aprovado pelo Comitê de ética no uso de Animais – CEUA da Faculdade de Odontologia de Araraquara – FOAr, UNESP.
n° 28/2020

UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"
Câmpus de Araraquara

FACULDADE DE ODONTOLOGIA

CERTIFICADO

Certificamos que a proposta intitulada *"CARACTERÍSTICAS FÍSICO-QUÍMICAS E BIOLÓGICAS DO CLÍNQUER COM DIFERENTES TAMANHOS DE PARTÍCULAS E DE CIMENTOS REPARADORES E OBTURADORES À BASE DE SILICATO DE CÁLCIO"* registrada com o nº **28/2020**, sob a responsabilidade do(a) **Prof(a). Dr(a). Mário Tanomaru Filho** – que envolve a utilização de dentes/ossos bovinos para fins de pesquisa científica – encontra-se de acordo com os preceitos da Lei nº 11.794, de 8 de outubro de 2008, do Decreto nº 6.899, de 15 de julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), e foi aprovado pela **COMISSÃO DE ÉTICA NO USO DE ANIMAIS (CEUA) DA FACULDADE DE ODONTOLOGIA DE ARARAQUARA** em reunião de 15/12/2020.

Finalidade	( ) Ensino (X) Pesquisa Científica
Vigência da autorização	Outubro/2023
Espécie/linhagem/raça	Dentes bovinos
Nº de animais	60
Peso/Idade	-
Sexo	-
Origem	Mondelli Indústria de Alimentos S/A

Profa. Dra. CARINA APARECIDA FABRICIO DE ANDRADE
Coordenadora da CEUA

Comissão de Ética no Uso de Animais - CEUA
Rua Humaitá nº 1.680 – Centro – CEP 14801-903 – Caixa Postal nº 331 - ARARAQUARA – SP
5º andar – fone (16) 3301-6459 / fax (16) 3301-6433 / e-mail: ceua.foar@unesp.br – home page: http://www.foar.unesp.br

ANEXO B – CERTIFICADO DO COMITÊ DE ÉTICA

Projeto de Pesquisa aprovado pelo Comitê de ética em pesquisa em seres humanos – CEP da Faculdade de Odontologia de Araraquara – FOAr, UNESP.

CAAE: 41926620.6.0000.5416

UNESP - FACULDADE DE ODONTOLOGIA - CAMPUS ARARAQUARA

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Características físico-químicas e biológicas do clínquer com diferentes tamanhos de partículas e de cimentos reparadores e obturadores à base de silicato de cálcio

Pesquisador: Mario Tanomaru Filho
Área Temática:
Versão: 2
CAAE: 41926620.6.0000.5416
Instituição Proponente: Faculdade de Odontologia de Araraquara - UNESP
Patrocinador Principal: CONSELHO NACIONAL DE DESENVOLVIMENTO CIENTIFICO E TECNOLOGICO-CNPQ

DADOS DO PARECER

Número do Parecer: 4.540.638

Apresentação do Projeto:
Trata-se apresentação do projeto de pesquisa cujo resumo consta: "O presente projeto de pesquisa é um estudo experimental ex vivo que tem como objetivo avaliar as propriedades físico-químicas de um novo cimento reparador (Bio-C Repair) e obturador (Bio-C Sealer) à base de silicato de cálcio. MTA Repair HP, Biodentine e AH Plus serão utilizados para comparação. Serão utilizados dentes humanos unirradiculares extraídos (N=54), provenientes do Banco de Dentes da Faculdade de Odontologia de Araraquara (FOAr-UNESP).Subprojetos 1: Avaliar por microtomografia computadorizada (micro-CT), o efeito do pH na alteração volumétrica e porosidade de cavidades retrógradas após obturação com Bio-C Repair, MTA Repair HP ou Biodentine (n=10), antes e após imersão em ácido butírico (pH 4.4) ou PBS (pH 10.4) por 7 dias. Subprojeto 2: Avaliar em canais circulares e achatados, por meio do micro-CT, o efeito do pH e espessura do cimento na interface guta-percha/cimento/dentina, alteração volumétrica e presença de vazios, dos cimentos obturadores Bio-C Sealer ou AH Plus (n=12), antes e após imersão em ácido butírico ou PBS em diferentes períodos. A bioatividade, bem como a interface gutapercha/cimento/dentina dos materiais serão também analisados em microscopia eletrônica de varredura (MEV). Os dados serão submetidos aos testes estatísticos apropriados (= 0,05)."

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Telefone: (16)3301-6459 **E-mail:** cep@foar.unesp.br

Objetivo da Pesquisa:

Objetivo Primário: Avaliar as propriedades físico-químicas de um novo cimento reparador e obturador à base de silicato de cálcio por meio de microtomografia computadorizada (micro-CT) e microscopia eletrônica de varredura (MEV). Objetivo Secundário: - Avaliar em micro-CT, o efeito do pH na alteração volumétrica e porosidade em cavidades retrógradas após obturação com cimentos reparadores Biodentine (Septodont), Bio-C Repair (BCR, Angelus) e MTA HP (Angelus), antes e após imersão em ácido butírico ou PBS por 7dias. - Avaliar em micro-CT, o efeito do pH e espessura do cimento na interface guta percha/cimento/dentina, alteração volumétrica e presença de vazios em canais radiculares achatados ou circulares após obturação com cimentos obturadores Bio-C Sealer (BCS, Angelus) ou AH Plus (AHP,Dentsply), antes e após imersão em ácido butírico ou PBS em diferentes períodos. A bioatividade, bem como a interface guta-percha/cimento/dentina dos materiais serão também analisados em MEV.

Avaliação dos Riscos e Benefícios:

Riscos: Os riscos envolvem os pesquisadores que serão submetidos a radiação uma vez que durante a seleção dos dentes será necessário realizar tomadas radiográficas para padronizar os espécimes, dessa maneira, será utilizado um sistema radiográfico digital (menor tempo de exposição) com utilização de uma caixa de chumbo que evitará a propagação da radiação e os envolvidos utilizarão avental de borracha plumbífero. No laboratório de Pesquisa Endodontia (Centro de confecção de amostras), os profissionais envolvidos farão uso de EPIs indicados quanto ao Mapa de Risco de cada ambiente.

Benefícios: Os benefícios obtidos desse estudo estão relacionados ao conhecimento das propriedades físico -químicas novos materiais reparadores e obturadores, permitindo seu uso adequado em diferentes situações clínicas.

Comentários e Considerações sobre a Pesquisa:

Trata-se de um estudo Nacional e unicêntrico, prospectivo, de caráter acadêmico realizado para obtenção de título de doutor.

O estudo inclui a participação do pesquisador principal e 1 doutorando, todos da FOAr.

O estudo irá utilizar 54 dentes humanos extraídos e provenientes do Banco de Dentes da Faculdade de Odontologia de Araraquara (FOAr-UNESP). O pesquisador declara que será realizada a devolução das sobras de material biológico.

Patrocinador CNPq.

A previsão de início é 01/03/2021 e encerramento em 15/08/2023.

O pesquisador solicitou dispensa da apresentação do TCLE, visto que o projeto irá utilizar dentes provenientes do Banco de Dentes Humanos da FOAr.

O projeto será desenvolvido nos seguintes Laboratórios:

1) Laboratório de Pesquisa em Endodontia – Centro de confecção de amostras (Departamento de Odontologia Restauradora);

2)Laboratório de Microtomografia do Departamento de Diagnóstico e Cirurgia;

3) Laboratório de Microscopia Eletrônica de Varredura.

Todos os laboratórios são da FOAr/UNESP.

Considerações sobre os Termos de apresentação obrigatória:

Documentos que foram inseridos na Plataforma Brasil:

1- PB INFORMAÇÕES BÁSICAS DO PROJETO

2- Declaração de autorização para uso do laboratório de pesquisa de endodontia

3- Declaração de autorização para uso do laboratório de microtomografia

4- Declaração de autorização para uso do Laboratório de Microscopia Eletrônica de Varredura.

5- Declaração de devolução de material biológico

6- Declaração de oferecimento de 54 dentes do banco de dentes da FOAr

7- Detalhamento do orçamento (despesas) do Projeto (no PB informações básicas do projeto)

8- Declaração de não ressarcimento

9- Projeto de doutorado completo

10- Termo de compromisso projeto

Recomendações:

Vide campo conclusões ou pendências e lista de inadequações.

Conclusões ou Pendências e Lista de Inadequações:

Sugere-se aprovação do projeto de pesquisa, visto que os pesquisadores atenderam a pendência de incluir o número da amostra no resumo do projeto. O pesquisador apresentou a carta resposta e incluiu o número da amostra em todos os arquivos pertinentes.

A nova redação do resumo ficou da seguinte forma: "...Serão utilizados dentes humanos unirradiculares extraídos (N=54), provenientes do Banco de Dentes da Faculdade de Odontologia de Araraquara (FOAr-UNESP). Subprojetos 1: Avaliar por microtomografia computadorizada (micro-CT), o efeito do pH na alteração volumétrica e porosidade de cavidades retrógradas após obturação com Bio-C Repair, MTA Repair HP ou Biodentine (n=10), antes e após imersão em ácido

butírico (pH 4.4) ou PBS (pH 10.4) por 7 dias. Subprojeto 2: Avaliar em canais circulares e achatados, por meio do microCT, o efeito do pH e espessura do cimento na interface gutapercha/cimento/dentina, alteração volumétrica e presença de vazios, dos cimentos obturadores Bio-C Sealer ou AH Plus (n=12)..."

Considerações Finais a critério do CEP:

Protocolo APROVADO em AD Referendum em 15 de fevereiro de 2021.

O pesquisador deverá encaminhar relatórios parciais a cada 01 (um) ano até o prazo final da pesquisa, quando deverá encaminhar o relatório final.

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_1671219.pdf	13/02/2021 17:29:51		Aceito
Outros	carta_resposta_.pdf	13/02/2021 17:23:50	Mario Tanomaru Filho	Aceito
Projeto Detalhado / Brochura Investigador	projeto_doutorado_.pdf	13/02/2021 17:23:03	Mario Tanomaru Filho	Aceito
Outros	termo_de_compromisso_m.pdf	09/01/2021 11:32:03	Mario Tanomaru Filho	Aceito
Outros	ressarcimento_de_gastos.pdf	04/12/2020 19:18:36	Mario Tanomaru Filho	Aceito
Outros	pesquisador_declaracao_aceite_lab_mev_.pdf	04/12/2020 19:17:42	Mario Tanomaru Filho	Aceito
Outros	documento_banco_de_dentes_.pdf	04/12/2020 19:17:03	Mario Tanomaru Filho	Aceito
Outros	devolucao_de_material_biologico_.pdf	04/12/2020 19:15:25	Mario Tanomaru Filho	Aceito
Outros	depesas_do_projeto_.pdf	04/12/2020 19:13:33	Mario Tanomaru Filho	Aceito
Outros	autorizacao_micro_ct_.pdf	04/12/2020 18:58:31	Mario Tanomaru Filho	Aceito
Outros	autorizacao_laboratorio_pesquisa_endodontia_.pdf	04/12/2020 18:56:44	Mario Tanomaru Filho	Aceito
Orçamento	orcamento_detalhado_.pdf	04/12/2020 18:54:07	Mario Tanomaru Filho	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	solicitacao_de_despensa_do_termo_de_consentimento_.pdf	04/12/2020 18:53:52	Mario Tanomaru Filho	Aceito

Folha de Rosto	folha_de_rosto_.pdf	04/12/2020 18:53:28	Mario Tanomaru Filho	Aceito

Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Não

ARARAQUARA, 15 de Fevereiro de 2021

Assinado por:
Andréa Gonçalves
(Coordenador(a))

Araraquara, 25 de março de 2024

Karina Ines Medina Carita Tavares