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PROPRIEDADES FÍSICO-QUÍMICAS E PIGMENTAÇÃO CORONÁRIA CAUSADA POR CIMENTOS BIOCERÂMICOS: ESTUDO *IN VITRO E EX VIVO*

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Tese apresentada como parte dos requisitos para obtenção do título de Doutora em Clínica Odontológica – Área de Concentração Endodontia.

Orientação: Profa. Dra.Roberta Kochenborger Scarparo

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RESUMO

O objetivo deste estudo foi avaliar as propriedades físico-químicas, a radiopacidade e a pigmentação coronária causada por cimentos biocerâmicos *in vitro e ex vivo*. Para as propriedades físico-químicas e radiopacidade os cimentos MTA Repair HP (MTA-HP), Biodentine (BD) e a nova formulação do MTA Ângelus Branco (MTA-Act), contendo tungstato de cálcio, foram comparados. A análise de pigmentação coronária foi realizada comparando os cimentos MTA-HP e MTA-Act com a formulação antiga do MTA Ângelus (MTA-Abo) com radiopatizante óxido de bismuto e com a a guta-percha (GP), na presença ou ausência de sangue e na presença ou ausência de adesivo dentinário para selamento da câmara pulpar. O tempo de presa inicial e final (n=7) foi determinado de acordo com a ASTM C266–15 e ISO 6876:2012. A solubilidade (n=11), o pH (n=10) e a liberação de ions cálcio (n=10) foram avaliados durante 28 dias, de acordo com a especificação da ANSI/ADA No. 57. A radiopacidade foi avaliada por meio do método proposto pela ANSI/ADA e do emprego de um simulador de tecidos (n=10). Para ambos os métodos, as amostras foram radiografadas ao lado de uma escala de alumínio, e as imagens analisadas no programa Adobe Photoshop para comparar valores de radiopacidade (em pixels) entre os materiais, com o terceiro degrau da escala de alumínio (mínima radiopacidade exigida pela ISO) e com a dentina. Para análise da pigmentação coronária, dentes monorradiculares(n=112) foram preparados para simular a morfologia de um dente com rizogênese incompleta, e os materiais foram empregados para a confecção de barreira cervical (n=7 por grupo/situação clínica simulada). As coordenadas que caracterizam a coloração dos dentes foram determinadas com um espectrofotômetro (Vita Easyshade 4.0, Vita Zahnfabrik, Bad Säckingen, Germany) e os índices CIELab, CIED 2000 e WID foram calculados para comparar as alterações de cor entre os grupos após 24, 30, 45 e 180 dias. Os resultados foram avaliados estatisticamente, considerando o nível de significância de 5%. O MTA-Act apresentou tempo de presa superior ao dos outros materiais ($p<0.05$). BD promoveu maior liberação de ions cálcio. Todos os materiais apresentaram pH alcalino e adequadas solubilidade e radiopacidade. Todos os materiais testados pigmentaram os dentes em um nível não aceitável. O uso de adesivo dentinário foi capaz de reduzir a pigmentação do MTA-Abo, mas apenas na ausência de sangue. Com base nesses resultados, pode-se concluir que os materiais biocerâmicos testados apresentam adequadas propriedades físico-químicas e radiopacidade. Entretanto, a pigmentação coronária segue sendo um desafio a ser superado.

Palavras-chave: Endodontia, Materiais biocerâmicos, Propriedades físico-químicas,Radipacidade, Pigmentação coronária

ABSTRACT

This study aimed to evaluate selected physical-chemical properties, radiopacity and tooth discoloration elicited by bioceramic materials *in vitro e ex vivo*. To evaluate physical-chemical properties MTA Angelus (MTA-Act), MTA Repair HP (MTA-HP) and Biodentine (BD) were compared. To assess tooth discoloration the recently modified White MTA Angelus containing calcium tunsgate (MTA-Act) and MTA Repair HP (MTA-HP) were compared to the original formulation of White MTA Ângelus (MTA-Abo) with bismuth oxide as the radiopacifier and with gutta-percha, both in the presence or abscence of blood and both in the presence or abscence of pulp chamber sealing with a dentin bonding agent. Initial and final setting times (n=7) were determined in accordance with ASTM C266–15 and ISO 6876:2012. Solubility (n=11), pH (n=10) and calcium íons release (n=10) were evaluated up to 28 days, in accordance with ANSI/ADA Specification No. 57. Radiopacity was assessed by two methods: ANSI/ADA method and Tissue Simulator method (n=10). For both methods, the materials were radiographed alongside with an aluminium stepwedge and the digital radiographs were analyzed in Adobe Photoshop determining the mean of grey scale pixel values of the materials, of the 3mm aluminium stepwedge and, in the tissue simulator method, of the dentin. To evaluate tooth discoloration, one-rooted (n=112) teeth were prepared to simulate the anatomical features of immature teeth and the bioceramic materials were used as coronary barrier endodontic materials. Color coordinates were obtained using a dental spectrophotometer (Vita Easyshade 4.0, Vita Zahnfabrik, Bad Säckingen, Germany) and CIELab, CIED 2000 and WID indexes were calculated to compare baseline tooth color with color measurements recorded after 24h, 30, 45 and 180 days. Tooth dicoloration was compared amongst the experimental groups in the four simulated clinical situations. Data obtained were statistically analysed and compared ($p<.05$). MTA-Act presented longer final setting time comparing to the other materials. All materials presented an alcaline pH. BD promoted greater calcium ions realese in most of the experimental periods. All materials presented adequate radiopacity in both methods evaluated. All the tested materials presented staining potential evaluated as unacceptable. In the absence of blood, the use of bonding agent in the pulp chamber allowed acceptable color changes in MTA-Abo samples. Beased on the present results, the bioceramic materials showed adequate physical-chemical propreties and radiopacity. However, tooth discoloration is still to be overcome.

Key-words: Endodontics, Bioceramic Materials, Physical-chemical properties,

Radiopacity, Tooth discoloration

SUMÁRIO

1. APRESENTAÇÃO

A presente Tese de Doutorado apresentou como objetivo a avaliação de propriedades físico-químicas, da radiopacidade e da capacidade de pigmentação coronária de materiais biocerâmicos. Foram realizados ensaios *in vitro* e *ex-vivo*, que serão apresentados da seguinte forma:

- Introdução
- Objetivos
- Artigo científico 1 – entitulado "**Physical-chemical properties and Radiopacity of three bioceramic cements**" e formatado de acordo com as normas do periódico Journal of Endodontics, fator de impacto 2.886 (Qualis A1 CAPES)
- Artigo científico 2 - entitulado "**Assessment of tooth discoloration caused by coronary barrier endodontic materials through CIELab, CIED 2000 and WID metrics**" e formatado de acordo com as normas do periódico Journal of Endodontics, fator de impacto 2.886 (Qualis A1 CAPES)
- Considerações finais

2.INTRODUÇÃO

O MTA foi introduzido na Endodontia em 1993, como material retrobturador apical e para reparo de perfurações radiculares (TORABINEJAD *et al*.,1993). Atualmente é considerado um padrão ouro para o tratamento dessas situações clínicas, sendo também empregado em tratamentos pulpares conservadores e para construção de barreiras apicais e de selamento cervical no tratamento de dentes com rizogênese incompleta. (MIN *et al*.,2008; KENNETH *et al*.,2013). O material, apresenta como vantagens o fato de ser bioativo e compatível, possuir dureza e solubilidade adequadas, promover vedamento efetivo, selando as vias de comunicação entre o sistema de canais radiculares e os tecidos circundantes. Além disso, ser radiopaco e induzir a formação de tecido duro, promovendo dentinogênese, osteogênese e cementogênese (ASGARY *et al*.,2005). Sua bioatividade deve-se ao potencial condutor e indutor de cementoblastos e osteoblastos, à capacidade de liberar componentes catiônicos e quimicamente similares à hidroxiapatita. (TINGLY *et al*.,2008; CAMILLERI *et al*.,2005). Além disso o material apresenta excelentes propriedades antimicrobianas, inibindo o crescimento de diversos microrganismos pelo seu elevado pH (WHATTS *et al*.,2007).

O MTA é um pó constituído de silicato de tricálcico, aluminato tricálcico, óxido de tricálcico, óxido de silicato, óxido de bismuto e ainda pequenas quantidades destes outros óxidos que modificam propriedades físico e químicas do material (TORABINEJAD *et al*., 1993). O MTA branco foi introduzido como um substituto para o MTA cinza, visando fornecer uma matriz mais parecida com a cor natural dos dentes. Esse material caracteriza-se pela exclusão dos componentes de ferro, obtendo partículas menores, melhorando sua manipulação e suas propriedades químicas (CAMILLERI *et al*.,2005). Estudos prévios demonstraram que o MTA branco apresenta propriedades físico-químicas e biológicas semelhantes às do MTA cinza convencional (MOTA *et al*.,2010).

Dificuldades relacionadas à manipulação do MTA e ao seu potencial de causar pigmentação dentária motivaram o desenvolvimento de técnicas e produtos alternativos. Dentre esses, podem ser citados o emprego de adesivo dentinário na câmara pulpar de dentes em regiões esteticas, e o desenvolvimento de materiais como o MTA Repair HP (Angelus, Londrina, Brasil), o Biodentine (Septodont,Saint-Maur,France) e a nova formulação do MTA Branco da Ângelus, substituindo o óxido de bismuto por Tungstato de cálcio(MOTA *et al*.,2010; OSKOEEl *et al*.,2014; YELAMALI,2016; ÂNGELUS,2018).

O MTA Repair HP é um material à base de silicato, desenvolvido recentemente visando manter propriedades biológicas e aprimorar propriedades físicas do MTA convencional. O pó do MTA Repair HP é composto principalmente por silicato tricálcico, silicato dicálcico, aluminato tricálcico, óxido de cálcio, carbonato de cálcio e tungstato de cálcio (radiopatizante), água e um agente plastificante. De acordo com o fabricante, este novo material tem alta plasticidade e propriedades físicas melhoradas, em comparação com o MTA branco, além de diminuir a capacidade de pigmentação dentária (ÂNGELUS, 2018). Um estudo realizado por Silva *et al* (2016), o MTA Repair HP mostrou maiores valores de resistência à tração em comparação ao MTA Ângelus branco. Além disso, a elevada plasticidade de MTA Repair HP pode melhorar a adaptação marginal do cimento com as paredes de dentina, aumentando a força de união entre o material e o dente.

O Biodentine é outra alternativa ao MTA, sendo composto de pó de silicato de tricálcico (71,7%), carbonato de cálcio (22,2%), óxido de zircônia como radiopatizante (6,1%) e silicato de dicálcio. O líquido contém cloreto de cálcio como acelerador de presa e um policarboxilato modificado como agente redutor de água (LAURENT, CAMPS ,2012).De acordo com estudos prévios, o Biodentine é um material bioativo que apresenta resistência a pigmentação superior ao MTA, citotoxidade semelhante ao MTA, estabilidade de cor e excelente capacidade de selamento.(VALLÉS *et al*., 2013; LAURENT, CAMPS ,2012). Rajasekharan et al (2014), em uma revisão sistemática, concluiu que considerando suas propriedades físicas e biológicas, o Biodentine pode ser uma alternativa eficiente em uma variedade de aplicações clínicas, tanto no campo da endodontia, como para

tratamento de traumas dento-alveolares, odontologia restauradora e odontopediatria. Ainda assim, ainda há pouca evidência clínica para apoiar todas as possíveis indicações.

Outra alternativa recentemente sugerida para reduzir o risco de pigmentação coronária em dentes tratados com MTA é o emprego de adesivos dentinários na câmara pulpar. Nesse sentido, o uso de adesivos evitaria a infiltracão marginal, impedindo que o MTA e o sangue penetrassem nos túbulos dentinarios. (JANG *et al* ,2013)

Ainda que a possibilidade de reduzir o risco de pigmentação coronária com a introdução de novos materiais e com o uso de adesivos dentinários sejam promissoras, é necessário avaliar se os novos materiais e técnicas propostos apresentam- se, de fato, efetivos. Além disso, é preciso verificar se as modificações na formulação dos materiais biocerâmicos não alteram as propriedades físico-químicas vantajosas do MTA, bem como se a sua radiopacidade permanece adequada para a prática clínica.

3.OBJETIVOS

3.1- Objetivo geral

Avaliar as propriedades físico-químicas, a radiopacidade e a capacidade de pigmentação dentária de cimentos biocerâmicos.

3.2- Objetivos específicos

- Avaliar propriedades físico-químicas, incluindo a solubilidade, os tempos de presa inicial e final, o pH e a liberação de íons cálcio promovida pelos cimentos MTA Repair HP (MTA-HP), Biodentine (BD) e pela nova fórmula do MTA Ângelus branco, contendo tungstato de cálcio.

- Avaliar a radiopacidade dos cimentos MTA-HP, BD e da nova fórmula do MTA-Angelus, contendo tungstato de cálcio.

- Avaliar a pigmentação coronária ocasionada pelos cimentos MTA-HP, pela antiga fórmula do MTA Ângelus branco (com óxido de bismuto) e pela nova fórmula do MTA-Angelus Branco (com tungstato de cálcio), Guta-percha (GP) na ausência ou presença de sangue e adesivo dentinario.

4. ARTIGO 1

Physicochemical properties and radiopacity of three bioceramic cements.

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Camila Zimmermann Rabello - Main researcher. Helped carry out the experiment, analyzed and interpreted the data, drafted the article, and approved the final version.
Lucenio João Macedo Ferri – Helped carry out the experiment and analyzed the data.
Bruna Signor - Helped carry out the experiment and analyzed the data.
Patrícia Maria Poli Kopper - Contributed to the conception and design of the manuscript and revised and approved the final version.
Fabiana Soares Grecca - Contributed to the conception and design of the manuscript and revised and approved the final version.
Lina Naomi Hashizume - Helped carry out experiment, analyzed and interpreted the data on the physicochemical properties of the materials.
Vania Regina Camargo Fontanella - Helped carry out experiment and analyzed and interpreted the data on radiopacity.
Roberta Kochenborger Scarparo – Study supervisor. Contributed to the conception and design of the manuscript, analyzed and interpreted the data, and revised and approved the final version.

Abstract

Introduction: This study aimed to compare the physicochemical properties and radiopacity of MTA Angelus (MTA-A), MTA Repair HP (MTA-HP), and Biodentine (BD). ***Methods*:** Baseline and final setting times (n=7) were determined in accordance with ASTM C266–15. Solubility (n=11), pH (n=10), and calcium ion release (n=10) were evaluated up to 28 days, in accordance with ANSI/ADA specification no. 57. Radiopacity was assessed by two methods: ANSI/ADA and the tissue simulator method. In the ANSI/ADA method (n=10), the materials were placed in standard rings and radiographed. In the tissue simulator method, polyethylene tubes filled with the material were placed in the root canal of a tooth in the tissue simulator and radiographed. In both methods, the specimens were radiographed using an aluminum stepwedge and the digital radiographs were analyzed in Adobe Photoshop, determining the mean grayscale pixel values of the materials, of the 3-mm aluminum stepwedge, and of the dentin, the latter of which performed on the tissue simulator. The data obtained from each test were statistically analyzed and compared ($p<.0.05$). ***Results:*** MTA-A presented longer final setting time compared to the other materials. There were no significant differences in the mass values of materials during the experiment. All materials presented an alkaline pH. BD promoted greater calcium ion release in most of the experimental periods. All materials presented adequate radiopacity in both methods. BD showed lower radiopacity than MTA-A and MTA-HP in the tissue simulator method. All groups presented higher radiopacity in the tissue simulator when compared to the ANSI/ADA method. ***Conclusions:*** MTA-A, MTA-HP, and BD showed adequate physicochemical properties and radiopacity, and were considered suitable to be used in clinical practice.

Key-words: Bioceramic materials, radiopacity, physical-chemical properties

Introduction

Mineral trioxide aggregate (MTA) had been initially developed for sealing root perforations and as a root-end filling material (1). Due to its physicochemical (1) and biological (2) properties, it has been also indicated for vital pulp therapy,(2) as an apical plug (3) and as a coronal barrier in the endodontic treatment of immature teeth(4). MTA formulation comprises a powder - containing tricalcium aluminate, tetracalcium aluminoferrite, calcium sulfate, gypsum, and bismuth oxide - and distilled water (5). White MTA Angelus (MTA-A) is slightly different from the original MTA, with a lower content of tetracalcium aluminoferrite and calcium sulfate (6). More recently, bismuth oxide has been replaced with calcium tungstate as radiopacifier to avoid tooth discoloration from the reaction of bismuth oxide with sodium hypochlorite and/or dentin collagen (7), but the physicochemical properties and radiopacity of the new formulation still has to be studied.

Besides the risk of tooth discoloration, difficulties with handling (8) have encouraged the development of alternative materials. MTA Repair HP (MTA-HP) (Angelus, Londrina, PR, Brazil) has been developed to provide higher plasticity. The main difference from the most recent formulation of MTA-A is the addition of an organic plasticizer to distilled water (9). Recent studies have demonstrated that MTA-HP improves resistance to dislodgement and flowability (9) maintaining the widely acclaimed biological properties of MTA (6). However, other physicochemical properties of this material, as well as its radiopacity, should be further investigated.

Biodentine™ (BD) (Septodont, Sair Maur de Fossés, France) is another alternative to MTA. It contains tricalcium silicate, dicalcium silicate, calcium carbonate, iron oxide, and zirconium oxide (powder), in addition to a liquid with calcium chloride (accelerator), water-soluble polymer, and a water-reducing agent (10). Greater microhardness and resistance to compressive loading are some of the advantages of BD in comparison to MTA (11.) Studies evaluating biological properties (12) and antimicrobial activity (13) have also indicated favorable properties. On the other hand, there is no consensus on the radiopacity of BD when the ANSI/ADA method (14,15) is used; therefore, additional methods that evaluate radiopacity under the influence of osseous, dental, and soft tissue superimposition should be considered (16). Previous studies have assessed the radiopacity of endodontic sealers using the tissue simulator (17), but to date, this method has not been used to evaluate bioceramic materials.

The present study aimed to compare baseline and final setting times, pH,

solubility, and calcium ion release of MTA-A, MTA-HP, and BD. Moreover, the radiopacity of these materials was evaluated both by the ANSI/ADA method and by the tissue simulator.

Materials and Methods

This study was approved by the local research ethics committee (protocol no. 3.007.591). Setting time, solubility, pH, release of calcium ions and radiopacity of MTA-A (Angelus, Londrina, Brazil), MTA-HP (Angelus, Londrina, Brazil), and BD (Septodont, Sair Maur de Fossés, France) were evaluated. All materials were prepared according to the manufacturers' instructions.

Setting time

The baseline and final setting times were evaluated according to ASTM C266–15 and ISO 6876:2012 (18). Plaster molds (n=7) with an internal diameter of 10 mm (± 0.1 mm) and a height of 2 mm (± 0.1 mm) were filled with the mixed material. The specimens were maintained for 5 min in an incubator at 37 °C with a relative humidity of 99 ± 5% before the baseline setting time measurements. Baseline setting time was measured with a Gillmore needle [diameter of 2 mm (± 0.1 mm), height of 5 mm, and weight of 100 g (± 5 g) carefully lowered onto the surface of the specimen without any further pressure. This procedure was repeated every 60 s until an impression was no longer visible on the material surface and the baseline setting time was then recorded. The final setting time (time elapsed from the beginning of mixing to the time at which no indentation was detected on the surface of the specimens) was determined with a larger Gillmore needle [diameter of 1 mm (± 0.1 mm) and weight of 456.5 g (± 5 g).

Solubility

The solubility test (n=11) was adapted from the American National Standard Institute/American Dental Association (ANSI/ADA) specification no. 57/2000(19) and ISO 6876:2012 (18). MTA-A, MTA-HP, and BD specimens were prepared using plastic molds with a height of 1.5 mm (± 0.1 mm) and an internal diameter of 7.75 mm (± 0.1 mm) (20). The molds were placed on a glass plate and filled with the mixed material. Another plate was positioned over the specimens and then stored in

an incubator at 37 °C with a 95% relative humidity, using a setting time three times longer than at baseline, as recommended by the manufacturers (12 min for MTA-A and MTA-HP, and 15 min for BD. The specimens were unmolded and weighed on a precision scale with an accuracy of 0.001g (Sartorius 1801MPS, Göttingen, Germany) to determine their initial mass (P-0). After the baseline weight measurements, the specimens were immersed in 15-mL Falcon tubes (Labor Import, Osasco, São Paulo, Brazil), filled with 7.5 mL of deionized water and maintained in the incubator (SXCR80, Sterilifer Ind. Com. Ltda., Diadema, Brazil) for 24 h. Subsequently, the specimens were removed from the incubator, slightly dried with absorbent papers, and placed in a drying chamber at 37 °C for 48 h. The specimens were then weighed in 72-hour shifts for 28 days. The specimens were maintained in the incubator throughout the weight measurements. Solubility was obtained by calculating the mass loss after the experimental periods in comparison to P-0.

pH and calcium ion release

For pH and calcium ion release analysis (n=10), MTA-Act, MTA-HP, and BD were inserted into 10 x 1.6-mm polyethylene tubes. After the baseline setting time (provided by the manufacturers), the specimens were inserted into 50-mL Falcon tubes (Cral Artigos para Laboratório Ltda, Cotia, São Paulo, Brazil.) containing 10 mL of deionized water (pH=7.4). The specimens were stored at 37°C during the experimental period. The storage water was replaced at each endpoint (1, 3, 12, and 24 h and 7, 14, 21 and 28 days), and the collected water was analyzed for pH by using a pH meter (Digimed, Digicrom Analitica, Campo Grande, Brazil), previously calibrated with standard solutions with known pH (4 and 7). The calcium ion release assessment was performed in the same experimental periods used for pH analysis. The calcium levels of the collected specimen were analyzed by the colorimetric method using Arsenazo III (21).

Radiopacity

The radiopacity of MTA-Act, MTA-HP, and BD was evaluated by two methods. In the ANSI/ADA method (n=10), the materials were prepared and placed in acrylic plates containing 4 x 1.5-mm rings. The filled rings were stored at 37 °C (±1) in 95% (±5) humidity for 7 days until the materials were completely set. Afterwards, the specimens were radiographed using a phosphor plate and an aluminum stepwedge.

Radiopacity was further evaluated using the tissue simulator developed by Gegler and Fontanella (16). Briefly, to build the simulator, the maxillary anterior region of a human skull was split by sagittal osteotomy into two segments fixed with wax (Wilson, São Paulo, Brazil) in a plastic container (length of 56 cm; width of 52.5 cm; depth of 53.5 cm). Distances of 1 cm were established between the external surfaces of the buccal and palatal segments and the container walls. This space was filled with self-curing acrylic (Artigos Odontológicos Clássico, São Paulo, Brazil) that could simulate the soft tissues. A distance of 0.5 cm was established between the internal surface of the buccal bone and the internal surface of the palatal bone. The space was filled with wax, which was used to fix a human canine root with the previously prepared root canal. The root was inserted up to the point at which the cementum-enamel junction coincided with the level of the alveolar crest. To evaluate radiopacity in this tissue simulator, the materials were manipulated and introduced into polyethylene tubes (length of 10 mm and diameter of 1.6 mm; Abbott Lab do Brasil, São Paulo, Brazil) with a syringe to avoid bubbles (n=12 for each cement). The filled tubes were stored at 37 °C (±1) in 95% (±5) humidity for 7 days until the materials were completely set. Thereafter, they were individually placed in the root canal of a canine tooth in the tissue simulator and radiographed using a phosphor plate and an aluminum stepwedge.

In both methods, the radiographs were obtained using a radiographic unit (Timex 70C, Gnatus, Ribeirão Preto, Brazil) operating at 70 kV and 10mA, with a 0.1s exposure time and a 30-cm focal distance set.The digital images were analyzed using Adobe Photoshop® CS5 (Adobe Systems, San Jose, CA, USA). For the images obtained with the ANSI/ADA method, a standard-size square (400 pixels) was drawn at the center of the standard disc (material), and another one was drawn in the third step, from left to right, of the aluminum stepwedge, equivalent to 3 mm of aluminum. In the simulator method, three standard-size squares (400 pixels) were drawn: one under the tube containing the material, another one under the dentin (both in the bone tissue overlap region), and the third one in the aluminum stepwedge at the same step described above. The mean and standard deviation of the grayscale pixel values of each selected area were measured and recorded using the histogram tool.

Data analysis

The statistical analysis was performed with GraphPad Prism 5.0 (GraphPad Software, San Diego, CA, USA) ($\alpha = 0.05$). Baseline and final setting times were

compared amongst the experimental groups using one-way ANOVA and Tukey's post-hoc test. The solubility of each material was evaluated throughout the experiment using repeated-measures ANOVA and Tukey's post-hoc test. Calcium ion release and pH were compared amongst the groups and the experimental periods by two-way ANOVA and Tukey's post-hoc test.

In both methods tested herein, radiopacity was compared amongst the groups using one-way ANOVA and Tukey's post-hoc test. The minimum radiopacity recommended by ANSI/ADA for the aluminum stepwedge (equivalent to 3 mm of aluminum) was used as a control for both methods. Besides, in the tissue simulator, dentin radiopacity was also used as a control. To compare the methods, the data were evaluated by Student's unpaired t-test.

Results

Baseline and final setting times of all tested materials are shown in Table 1. MTA-A presented a longer final setting time compared to MTA-HP and BD ($p = 0.0001$). There was no significant difference when evaluating the variability of mass values in the solubility test ($p<.05$) (Table 1). All the tested materials presented an alkaline pH - close to 10 - during the experimental period. After 1 h, MTA-HP presented a more alkaline pH in comparison to MTA-A ($p<0.05$). Both MTA-HP and MTA-A showed significantly greater calcium ion release after 21 days, while BD presented greater release of calcium ions from the third day ($p<0.05$). BD promoted greater calcium ion release in most of the experimental periods, except after 14 and 28 days ($p<0.05$).

Table 2 summarizes the findings on radiopacity. All tested bioceramic cements showed higher radiopacity in the tissue simulator method as compared to the ANSI/ADA method ($p<0.05$). In both methods, all materials presented higher radiopacity than 3 mm of aluminum stepwedge. In the tissue simulator, the three tested materials presented higher radiopacity than dentin. There were no significant differences amongst the materials when evaluating radiopacity through the ANSI/ADA method, while BD showed lower radiopacity than MTA-A when materials were evaluated in the tissue simulator ($p<0.05$).

Discussion

In the present study, the physicochemical properties and the radiopacity MTA-A, MTA-HP, and BD were investigated. Baseline setting time was evaluated to observe the suitability of the tested materials in clinical procedures performed in a single appointment, since a faster setting time reduces dislodgement after material placement (22). The original MTA set after 2 h and 45 min (1) which has traditionally been considered a drawback. In agreement with previous studies (9,23) the three tested materials showed adequate baseline setting time - about 20 minutes – which was similar among the materials. Modifications in the original formulation, such as the absence of calcium sulfate (24) and bismuth oxide (9) have probably influenced this outcome.

With regards to the new formulation of MTA-A, although the values of baseline setting time were very similar to those found in other studies that have evaluated MTA-A with bismuth oxide (9,23) the final setting time was longer in comparison with that of the other materials, probably due to differences in the formulations of the materials. MTA-HP differs from MTA-A mainly in the liquid component. The organic plasticizer of MTA-HP could have altered the water content after the mixing process, thus affecting setting time. In this regard, setting time is directly affected by moisture (25). Moreover, higher surface area has been previously observed for MTA-HP in comparison with MTA-A, which correlates with smaller particle sizes and could accelerate the setting time (26). In BD, the addition of calcium chloride in the liquid component has probably reduced the setting time (27). Accordingly, it has been previously observed (28) that the use of calcium chloride is efficient in accelerating the setting of bioceramic materials.

Solubility and contamination before complete setting are concerns for materials with a longer final setting time (22). On the other hand, the differences observed herein did not seem to affect MTA-A solubility. As a matter of fact, there were no significant differences in solubility amongst the materials throughout the experiment. The three silicate-based cements showed constant mass values and presented adequate behavior - in accordance to ISO 6876 (19) - proving their suitability as root repair materials, when in contact with body fluids. Calcium tungstate is insoluble in water, contributing to MTA-A and MTA-HP insolubility (6).

An ideal bioceramic material should have alkaline pH and calcium ion release as early as possible, and these enviromental conditions should be maintained for long periods. In this regard, an alkaline pH should contribute to the maintenance of an environment that is inhospitable to microbial growth (22,25) and, together with the

presence of calcium ions, favor the mineralization process (12,13). In the current study, MTA-HP showed greater pH than MTA-A after 1 h, but all the bioceramic materials sustained an alkaline pH throughout the 72-hour experimental period. BD showed a higher release of calcium ions earlier than did the other materials, also producing higher levels of ion release in most of the experimental periods. This feature could have an effect on previously described positive aspects of BD, such as good biocompatibility, bioactivity and biomineralization (29-30). In this regard, the ability of BD to promote pulp mineralization in shorter periods than the other bioceramic materials has been suggested in an entire human tooth culture model (10).

Among other characteristics, an ideal bioceramic material should be more radiopaque than dentin and tooth surrounding structures. In the current investigation, two methods for evaluation of radiopacity were compared, showing that under the influence of tissue superimposition, bioceramic materials presented significantly higher radiopacity than when they were evaluated through the ISO recommended method. Accordingly, a previous study (31) has compared the radiopacity of endodontic sealers using the same tissue simulator used herein, but for hard and soft tissues, showing higher radiopacity than that of the method recommended by the ISO.

Previous studies evaluating the radiopacity of bioceramic materials have used only the ISO recommended method. In agreement with the current outcomes, Guimarães *et al.* (6) showed that MTA-HP meets the criteria recommended by ISO 6876:2012 (19), presenting similar radiopacity when compared to MTA-A. Note that, in that study, as well as in the other investigations on MTA Angelus radiopacity (15,32), bismuth oxide was still used as radiopacifier. The current outcomes revealed that calcium tungstate was able to maintain suitable radiopacity of MTA-A in both methodologies used.

Conflicting results have been observed for BD radiopacity in previous investigations, which ranged from suitable (14,33) to inadequate according to ISO (34). The current results show that BD met the ISO criteria and, although it presents lower radiopacity compared to the other materials, the differences are not significant. The tissue simulator method employed herein has allowed evaluating radiopacity closer to what is observed clinically. In this regard, under the influence of tissue superimposition, radiopacity tends to be higher, confirming that BD can be employed in endodontic practice. Although BD radiopacity in the tissue simulator is significantly lower than that of MTA-A and MTA-HP, these differences probably are not clinically relevant, since dentin presented lower radiopacity than did BD (15,34).

In conclusion, MTA-A, MTA-HP, and BD showed adequate physicochemical properties and radiopacity and can thus be used in clinical practice.

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Tables

Table 1. Mean and standard deviation (±SD) of baseline and final setting times (minutes), solubility expressed as mass loss (%) in relation to baseline mass (g), pH and calcium ions release values of MTA HP, BD, and MTA-A measured in different experimental periods.

		MTA-HP	BD	MTA-A
Setting time	Baseline (min)	19.14 ± 1.46^{Aa}	21.71 ± 1.60^{Aa}	18.71 ± 0.95^{Aa}
	Final (min)	35.86 ± 2.19^{Aa}	33.29 ± 4.31^{Aa}	44.42 ± 2.81^{Ab}
Solubility	m-0	0.16 ± 0.01^{Aa}	0.17 ± 0.02^{Aa}	0.18 ± 0.01^{Aa}
	m-1	0.17 ± 0.01^{Aa}	0.15 ± 0.05^{Aa}	0.20 ± 0.22^{Aa}
	m-2	0.17 ± 0.01^{Aa}	0.16 ± 0.01^{Aa}	0.20 ± 0.22^{Aa}
	m-3	0.16 ± 0.05^{Aa}	0.16 ± 0.01^{Aa}	0.20 ± 0.02^{Aa}
	m-4	0.17 ± 0.01^{Aa}	0.16 ± 0.01^{Aa}	0.20 ± 0.02^{Aa}
	m-5	0.17 ± 0.01^{Aa}	0.16 ± 0.01^{Aa}	0.20 ± 0.02^{Aa}
	m-6	0.17 ± 0.01^{Aa}	0.16 ± 0.01^{Aa}	0.19 ± 0.02^{Aa}
	m-7	0.17 ± 0.01^{Aa}	0.16 ± 0.01^{Aa}	0.19 ± 0.02^{Aa}
pH	1h	10.11 ± 0.4^{Aa}	10.14 ± 0.14^{Aab}	9.82 ± 0.10^{Ab}
	3h	10.28 ± 0.13^{Ba}	10.50 ± 0.44^{Aa}	10.31 ± 0.09^{Ba}
	12h	$10.16 \pm 0{,}25^{ABCa}$	10.26 ± 0.46^{Bba}	$10{,}28 \pm 0{,}21^{BCa}$
	24h	10.20 ± 0.34^{ABa}	10.19 ± 0.60^{ABa}	10.27 ± 0.27^{BCa}
	7 days	10.33 ± 0.50^{ABa}	10.52 ± 0.62^{ABba}	10.55 ± 0.20^{Da}
	14 days	10.68 ± 0.41^{ABCDa}	10.87 ± 0.64^{ABba}	10.99 ± 0.1^{Ea}
	21 days	10.86 ± 0.58^{Da}	10.83 ± 0.74^{Aba}	10.92 ± 0.28^{Ea}
	28 days	10.87 ± 0.54^{Da}	10.79 ± 0.74^{ABa}	10.76 ± 0.45^{BCDEa}
Calcium ion release	1h	335.63 ± 102.56^{Aa}	483.70 ± 30.77^{Ab}	397.95 ± 104.75^{Aab}
	3h	371.46 ± 24.70^{Aab}	384.34 ± 11.34^{BCa}	328.66 ± 42.13^{Ab}
	12h	349.96 ± 34.05^{Aa}	383.19 ± 7.16^{BCb}	337.15 ± 23.14^{Aa}
	24h	333.08 ± 48.43^{Aa}	422.70 ± 5.44^{Cb}	329.48 ± 51.54^{Aa}
	7 days	400.28 ± 26.40^{Ba}	433.84 ± 8.07^{Db}	403.65 ± 20.73^{Ba}
	14 days	482.38 ± 41.51^{ABa}	421.55 ± 7.15^{Eb}	414.22 ± 9.39^{Bab}
	21 days	573.56 ± 18.77^{Ca}	591.95 ± 2.07^{Fb}	576.73 ± 23.26^{Cab}
	28 days	570.87 ± 51.79^{Ca}	579.83 ± 50.31^{Fa}	559.55 ± 60.30^{Ca}

Values whih the superscript capital letters were not statistically different when comparing the same material throughout the experimental periods. Different superscript lower case letters represent signification differences among the tested materials ($p<0.05$).

Table 2– Mean and standard deviation of radiopacity (in grayscale pixel) of MTA-HP, BD, and MTA-A (n=10 per group). Values with different superscript uppercase letters represent statistically significant differences between the methods. Values with the same superscript lowercase letters were not statistically different when comparing the groups ($p < 0.05$).

	ANSI/ADA		**Tissue Simulator**		
	Material	*AL*	*Material*	*AL*	*Dentine*
MTA-HP	91.95 ± 17.81^{Aa}	83.50±10.01^{Ab}	192.5 ± 1.75^{Bab}	89.12±0.88^{Ac}	181.17±1.05^{d}
BD	94.38 ± 5.12^{Aa}	85.50±10.00^{Ab}	191.1 ± 3.21^{Ba}	99.35±0.90^{Ac}	180.10±0.88^{d}
MTA-A	98.35 ±12.9^{Aa}	88.10±09.34^{Ab}	194.3 ±1.37^{Bb}	97.13±0.78^{Ac}	182.68±0.98^{d}

5. ARTIGO 2

Assessment of tooth discoloration caused by coronary barrier endodontic bioceramic materials through CIELab, CIED 2000 and WID metrics

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Abstract

Introduction: The aim of this study is to assess tooth discoloration caused by coronal barrier endodontic bioceramic materials either in the presence or abscence of blood and either using or not a dentin-bonding agent.

***Methods*:** Eighthy (112) human maxillary and mandibular single rooted teeth had their length and enamel-dentin thickness of crown buccal surface standardized. Root canals were prepared to simulate the morphology of immature teeth, and the sample was randomily divided according to the simulated clinical condition and to the material used for coronary barrier (n=7). Thus, the root canals were filled either with sheep blood or sterile saline, nearly 3 mm below the cementoenamel junction, and, in half of the specimens, a dentin bonding agent was used to seal the internal walls of the pulp chamber. The recently modified White MTA Ângelus containing calcium tunsgate (MTA-Act) and MTA Repair HP (MTA-HP) were compared to the original formulation of White MTA Ângelus (MTA-Abo) with bismuth oxide as the radiopacifier and with gutta-percha (GP) in the presence or abscence of blood and in the presence or absence of pulp chamber sealing. Color coordinates were obtained using a dental spectrophotometer (Vita Easyshade 4.0, Vita Zahnfabrik, Bad Säckingen, Germany) and CIELab, CIEDE 2000 and WID indexes were calculated to compare baseline tooth color (T0) with color measurements recorded after 24h (T1), 30 (T2), 45 (T3) and 180 days (T4). Tooth dicoloration assessed both through CIE Lab and CIEDE 2000 metrics was compared amongst the experimental groups in the four simulated clinical situations. Kruskal-Wallis and Dunn tests were used to identify the differences between groups ($p<0.05$). Moreover, the WID index was described to evaluate if the color changes, before or after treatment, was whiter or darker.

Results: In the absence of blood and bonding agent, MTA-Abo and GP promoted lower color alteration in comparison to MTA-Act and MTA-HP ($p<0.05$). In the absence of blood, the use of bonding agent in the pulp chamber allowed acceptable color changes in MTA-Abo samples.

Conlcusions: All the tested bioceramic materials presented potential for tooth discoloration, regardless of bismuth oxide replacement for calcium tungstate. Further studies should be performed to improve materials composition, aiming to reduce their staining potential.

Key-Words: Endodontics, bioceramic materials, tooth discoloration, boding agent

Introdution

Pulp necrosis of immature permanent teeth is commonly caused by dental trauma and caries (1) and often affects anterior teeth. Within this context, revascularization procedures has been considered a promising alternative, allowing the continuity of root formation and, hence, tooth strengthening (2,3,4). According to the American Association of Endodontists (AAE), the treatment protocol consists of root canal chemical disinfection, promotion of bleeding into the root canal, and placement of a coronal barrier material over the formed blood clot (5).

White mineral trioxide aggregate (MTA) has been commonly used as a coronal barrier (6) due to its physico-chemical (7,8,9) and biological characteristics (9). However, it has been associated with tooth discoloration, probably induced by the radiopacifier component, bismuth oxide (10,11,12). MTA staining potential can be even higher in contact with blood (13), which is a concern in revascularization procedures. The use of a dentin-bonding agent on pulp chamber dentin has been suggested to mitigate tooth discoloration (14).

Recently, White MTA Ângelus (Angelus, Londrina, PR, Brazil) have changed its formulation by replacing the radiopacifier bismuth oxide by calcium tungstate. MTA Repair HP (Angelus, Londrina, PR, Brazil) added an organic plasticizer to the distilled water and also uses calcium tungstate to provide radiopacity (15). The manufacturer suggests that these modifications on the MTA formulation prevent tooth discoloration. A previous study in bovine teeth observed higher tooth discoloration caused by White MTA Ângelus in comparison to MTA Repair HP (16). However, the new formulation of this material, free of bismuth oxide, was not evaluated, and the presence of blood, as well as the use of a dentin-bondig agent was not considered.

Currently, studies that investigate tooth discoloration caused by endodontic materials (11,16,17) use the CIELab color notation system, evaluating parameters such as lightness and color gradients spanning green to red, and blue to yellow. The CIELab metric has been classically the standard parameter for total color difference between two objects, and consider that the greater the value, the larger the color difference and the more perceptible the difference to the human eye (18,19). More recently, aiming to control changes in the magnitude of tolerance judgments and to adjust for scaling of acceptability rather than perceptibility (19), the CIE recommended the use of CIEDE2000 color-difference formula (20,21). CIEDE2000 incorporates corrections for non-uniformity of CIELAB color space, a rotation term

that accounts for the interaction between chroma and hue differences in the blue region, and a modification of one of the coordinates of CIELAB that mainly affects colors with low chroma (22) and parameters accounting for the influence of illuminating and vision conditions in color difference analysis (18). Also recently, a new whiteness index (WID), based on the CIELab color notation system, was designed to enable the identification of whiteness differences among adjacent specimens (19). In this regard, WID provides not just answers regarding the magnitude of color changes, but also shows which value, before or after treatment, is whiter or darker (23).

Considering that previous studies on visual judgments observed advantages to the use of CIEDE2000 (21) and WID (19), several investigations in the dentistry and prosthodontics fields have used these metrics to provide more reliable results (23,24). However, to date, it has not been used to evaluate tooth discoloration caused by coronary barrier endodontic materials.

The aim of this study is to assess tooth discoloration caused by coronal barrier endodontic materials - either in the presence or abscence of blood and either sealing or not the dentin with a dentin-bonding agent - through CIELab, CIEDE2000 and WID indexes. The recently modified MTA Ângelus containing calcium tungstate (MTA-Act) and MTA Repair HP (MTA-HP) were compared to the original formulation of White MTA Ângelus (MTA-Abo) with bismuth oxide as the radiopacifier and with gutta-percha (GP).

Materials and methods

Sample Preparation

This study was approved by the local Ethics in Research Committee (protocol no. 3.007.591). One hundred twelve (112) human maxillary and mandibular single rooted incisors, canines and premolars planned for extraction were used. Teeth free of caries, restorations, cervical lesions and coronal discoloration were selected. The teeth were cleaned to remove debris and extrinsic stain, and then stored at 37^{o}C sterile saline solution (LBS, São Paulo, Brasil).

The apical portion of the roots was resected perpendicular to their long axis to standardize the teeth length in 15 mm. The apical openings of the roots were sealed using sticky wax (Wilson,São Paulo-SP ,Brazil) and the teeth were included in acrilic resin Filtek Z 350 (XT-3M ESPE, São Pulo,Brazil) up to the cementoenamel junction.

Endodontic access was performed under water colling using round diamont points (KG-sorensen, Cotia -São Paulo, Brasil) in high speed, and root canals were explored with a #10 K-Flexofile (Dentsply mallefer,Ballaigues,Switzerland) and prepared using #1- 4 Gates-glidden drills (Dentsply mallefer,Ballaigues,Switzerland). Irrigation with 5 ml of 2.5% NaOCl was performed after each bur preparation, followed by a final rinse with 5ml of 17% ethylenediaminetetraacetic acid (EDTA) for 5 minutes and of 1 ml of saline solution. In the buccal surface of teeth crown, enamel-dentin thickness was standardized to 3 mm using a digital caliper.

The recently modified White MTA Ângelus containing calcium tungstate (MTA-Act), MTA Repair HP (MTA-HP), the original formulation of White MTA Ângelus (MTA-Abo) with bismuth oxide as the radiopacifier and gutta-percha (GP) were compared regarding their potential to cause tooth discoloration in four simulated clinical situations. For each material, tooth discoloration was tested either in the presence or abscence of blood and either sealing or not the dentin with a bonding agent. The teeth were randomly assigned according to the coronal barrier material and clinical situation (n=7 per material/clinical situation).

The root canals were filled either with sheep blood (Labsul Produtos para Laboratorio, Porto Alegre, RS, Brazil) or sterile saline (LBS,São Paulo, Brazil), nearly 3 mm below the cementoenamel junction, using an endodontic syringe (Injex,São Paulo ,Brazil). A collagen barrier membrane (Gen Derm P, Baumer, São Paulo Brazil) was placed over canal content. For specimens in which the dentin bonding agent was used, the internal walls of the pulp chamber were sealed according to the manufacturer's instructions as follows: after the inner surfaces of the pulp chamber was etched for 15 seconds with 35% phosphoric acid (Vococid; Voco GmbH, Indian Land, USA), the dentin bonding agent (Solobond M; Voco GmbH, Indian Land, USA) was applied to the etched surfaces and cured for 20 seconds. All materials were mixed according to manufacturer instructions and placed into the root canal cervical portion below the CEJ, creating a 3-mm thickness coronal barrier. The materials were allowed to set, the remaining access opening of each tooth was filled with a composite restoration by using Filtek Z350 (3M ESPE, Stpaul, MN), and the specimens were stored at 37^{o}C sterile saline solution (LBS, São Paulo, Brazil).

Tooth Discoloration Assessment

Color coordinates were obtained using a dental spectrophotometer (Vita Easyshade 4.0, Vita Zahnfabrik, Bad Säckingen, Germany). Measurements were performed by a blind single trained operator using a gray background Flexipalette

Color Match (Smile line, St-Imier, Switzerland) and at a dental clinic with standardized D65 light illumination. At the beginning and after each measurement, calibration was performed as recommended by the manufacturer. The active point of the spectrophotometer was placed on the middle third of the coronal facial surface of each tooth measuring it for three times, which were averaged. The mean of baseline measurements was recorded after tooth preparation and before coronal barriers placement (T0). Other measurements were recorded after 24 hours (T1), 30 days (T2), 45 days (T3) and 180 days (T4).

CIELab color coordinates were used to calculate color changes, according to the following equation, where L epresents lightness axis, a the red-green axis, b the yellow-blue axis, and C the chroma.

$$\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2} .$$

Color difference was also assessed through CIEDE2000 ($\Delta E00$) formula, according to the following equation, where $\Delta L0$, $\Delta C0$, and $\Delta H0$ are the differences in lightness, chroma, and hue, respectively, for a pair of specimens. The weighting functions (S_L, *Sc* and S_H) adjust the total color difference for variation in the location of the color difference pair in *L', a' , b'* coordinates. The parametric factors (K_L, K_C and K_H) are correction terms for experimental conditions. Finally, a rotation function (*Rt*) accounts for the interaction between chroma and hue differences in the blue region.

$$\Delta E00 = [(\Delta L^2/K_L S_L)^2+(\Delta C^2/K_c S_c)^2+(\Delta H^2/K_H S_H)^2+R_T(\Delta C^2/K_c S_c)^2+(\Delta H^2/K_H S_H)]^{1/2}$$

The whiteness index for Dentistry (WID), which is based on CIELab coordinates, was calculated as the following equation, where higher WID values indicate whiter samples, whereas lower WID values (including negative values) indicate darker samples.

$$WI_D = 0.511L^* - 2.324\,a - 1.100b$$

The ΔE^*, $\Delta E00$ and ΔWI_D values were calculated to determine color differences between T1, T2, T3 and T4 in comparison to the baseline evaluation (T0).

CIELab and CIEDE 2000 color differences were finally evaluated with the most recent published data about 50%:50% acceptability (AT = 2.66 ΔE* ab units and 1.77 ΔE00 units) thresholds. These visual thresholds values were recently accepted by ISO. (ISO, 2016) Difference in WID were finally evaluated with the whiteness 50%:50% perceptibility (WPT = 0.61 ΔWID units) and 50%:50% acceptability (WAT = 2.90 ΔWID units) thresholds, which were obtained by a population of lay-people (18).

Statistical Analysis

Changes in CIELab, CIEDE2000 and WID metrics were compared amongst the experimental groups in each one of the four simulated clinical situations. Statistical analysis was performed by using nonparametric Kruskal-Wallis and Dunn tests ($p < 0.05$), as a result of the absence of normal distribution.

Results

In the absence of blood and bonding agent, CIEDE 2000 index revealed that MTA-Abo and GP presented lower values in comparison to MTA-Act and MTA-HP in all experimental periods ($p<0.05$), and that GP promoted less teeth staining than MTA-Abo in the interval T0-T4. For CIELab index, both GP and MTA-Act presented lower values in comparison to MTA-HP in all evaluated intervals, and GP showed lower tooth staining than MTA-Act at the interval T3-T0 ($p<0.05$). MTA-Act and MTA-HP presented less acceptable color changes compared to the other groups. WID also showed that MTA-Abo promoted more acceptable teeth discoloration (Figure 1).

When bonding agent was placed in the pulp chamber of teeth filled with saline, CIELab index was lower for GP compared to MTA-Act at T1-T0 ($p<0.05$). MTA-Act CIEDE 2000 index was higher than all other groups at T1-T0 and than MTA-Abo at T3-T0 ($p<0.05$). The WID index revealed more acceptable color changes for MTA-Abo and GP groups (Figure 2).

All samples filled with blood presented color changes, including those of the control group. Significant differences were not observed amongst the materials staining potential evaluated both by CIELab abd CIEDE 2000 indexes. WID revealed that samples in which GP was used as a coronal barrier showed higher darkening compared to the groups sealed with one of the bioceramic materials (Figure 3).

In the presence of blood, the use of bonding agent was not effective in reducing tooth discoloration. Both CIELab and CIEDE 2000 indexes did not show significant differences amongst groups ($p<0.05$), but WID index revealed that MTA-Act and MTA-Abo showed more acceptable changes in comparison to MTA-HP and GP, which tended to promote samples darkening (Figure 4).

Discussion

Crown discoloration is reported as common complication of revascularization procedures (13). This study evaluated staining potential of bioceramic materials used as coronal barrier in an *ex vivo* model simulating imature teeth.

Visual thresholds supplement descriptive and analytical statistics and are of paramount importance for interpreting color differences and for evaluating whether color differences are acceptable or not. CIELab is used in most of the studies in the endodontic field (16,25,26), but CIEDE 2000 and WID have been recently used in dental research for bleaching efficacy, comparing between visual and instrumental shade matching, dental shade guides, and other areas related to color compatibility, color stability, and color interaction (19,23). The present outcomes did not show major differences comparing CIELab and CIEDE 2000 metrics, probably because teeth discoloration elicited by the tested protocols was undoubtedly unacceptable to the human eye, and thus, both methods were sensitive to detect color differences.

WID index added additional information to the other metrics, since it diferentiate samples whitening or darkening (23). In this regard, most of the specimens tended to darken. However, some samples presented whiteness values after treatment. As previously suggested (27), restorative materials may act as an opatizer, reducing discoloration, which might have influenced color change in some teeth.

Most of the previous investigations on tooth discoloration used bovine teeth instead of human ones, revealing the staining potential of bismuth oxide in contact with dentin collagen and sodium hypochlorite (16,25,26). Moreover, these studies suggested that calcium tungstate does not cause dentin staining. In the present study MTA-Abo - that uses bismuth oxide as the radiopacifier - promoted teeth discoloration(25,26), but it was lower in comparison to that observed for MTA-HP and MTA-Atc groups - in which calcium tungstate is employed.

A possible explanation to the divergent results is related to the methods used to assess the staining potential of the materials. The current investigation was the first to observe to observe tooth discoloration elicited by MTA-HP and MTA-Act in a human teeth model simulating immature root development. Previous investigations comparing other materials in human teeth have also disagreed with the results of studies using the bovine model to assess teeth discoloration, suggesting that the differences between human and bovine dentin substrates could affect staining potential (28). It is recognized that morphological, chemical composition and physical property differences between the two substrates must be considered when interpreting results obtained from any experiment using bovine dentin (29). As a matter of fact, the number of tubules is higher in bovine specimens compared with human dentin (30) which might influence the penetration of the materials in the dentin.

Besides the differences on dentin substrates, divergent results may arise from other features of the studies model. While other investigations used dentin discs to assess the interaction of bioceramic components with dentin collagen (16,25,26), the method employed herein attempt to more closely simulate a revascularization procedure. Thus, human teeth were prepared aiming to simulate the morphology of an immature roots, coronal access preparation was performed, the bioceramic materials were placed in the cervical portion of roots as recommended (5), and teeth were then restored with a composite resin.

A previous investigation showed that MTA-HP presents significantly higher flow when compared to its predecessor White MTA Ângelus, containing bismuth oxide (31). In this regard, flow ability may also have favored the material penetration into dentin tubules and thus affected tooth discoloration.

As well as observed previously (32), in the absence of blood, the use of a dentin-bonding agent applied to the dentinal walls of the pulp chamber was capable to effectively reduce endodontic materials induced coronal discoloration. In this simulated clinical scenario, MTA-Abo differences in the CIELab, CIEDE 2000 (at T3-T0 interval) and WID (in all intervals) indexes before and after treatment were within an acceptable level to the human eye. This might be related to the sealing of dentin tubules with the bonding agent, leading to prevention of materials components migration to the tooth structure. A possible explanation for WID be more sensitive in observing acceptability at T1-T0, T2-T0 and T4-T0 intervals is that it considers an acceptable color differences for both whiteness and darkness.

In the blood groups, unacceptable color differences elicited by treatment were observed for all materials, regardless of the use of dentin bonding agent. Other

investigations have also demonstrated greater discoloration in teeth filled with blood (6), which probably is related to the proximity of blood inside the cervical portion of root canal with the cervical third of the crown, favoring the penetration of erythrocytes into the coronal tooth structure. Moreover, higher tooth discoloration could be attributed to color changes of bioceramic materials when exposed to blood. In this regard, it has been shown that blood contamination exacerbated discoloration induced by calcium silicate-based materials containing bismuth oxide or other radiopacifiers (33.34). Also in the presence of blood, WID index showed a greater tendency to darkening in the GP and MTA-HP groups. Probably, poor sealing provided by GP and the more plastic consistency of MTA-HP have favored blood contact with the cervical dentin, thus affecting teeth discoloration. Moreover, MTA-Abo and MTA-Act present hygroscopic ability (35), which could have contributed to reduce the amount of blood in contact with coronal dentin.

Conclusion

Under the conditions of this study, all the tested bioceramic materials presented potential for teeth discoloration, regardless of bismuth oxide replacement for calcium tungstate. The use of a dentin bondig agent was only effective to mitigate color changes elicited by MTA-Abo in the abscence of blood. Thus, the tested materias should be used with caution in revascularization procedures in teeth located in an esthetically important area. Further studies should be performed to improve materials composition, aiming to reduce their staining potential. Since it considers both whiteness and darkness of samples, WID appeared to be more sensitive in observing color differences acceptability and should be considered in future investigations evaluating teeth discoloration.

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Figure Legends

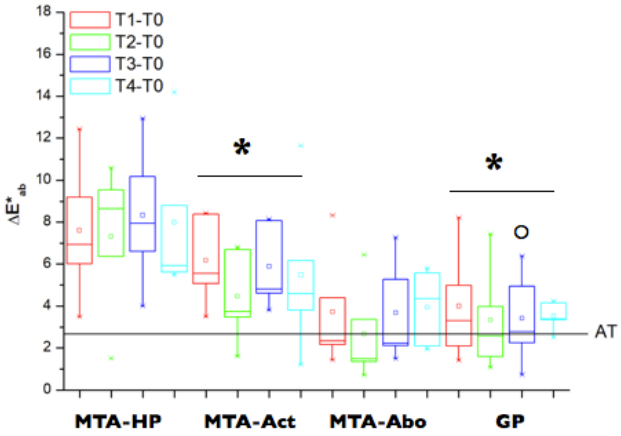
Figure 1. Median and SD values of color differences in samples filled with saline, using (A) ΔE* ab (B) ΔE00 and ΔWID (C) metrics, comparing the baseline indexes (T0) with 24 hours (T1), 30 days (T2), 45 days (T3) and 180 days (T4) after coronal barrier application. Values below AT indicate adequate acceptability threshold. GP and MTA-Act showed significantly lower ΔE*ab values compared to those of MTA-HP in all experimental periods (*p<0.05); GP promoted lower ΔE*ab values compared to MTA-Act at T3-T0 interval (^{O}p<0.05). ΔE00 index was significantly lower for MTA-Abo and GP in comparison to MTA-HP and MTA-Act in all experimental periods (*p<0.05); GP presented lower ΔE00 values at the interval T4-T0 (^{O}p<0.05). For ΔWID values, positive values indicate whitening, while negative values indicate darkening. Differences within 0-2.5 represents acceptable color changes, while greater values represent perceptible and unaceptable tooth discoloration.

Figure 2. Median and SD values of color differences in samples filled with saline after dentin bonding agent application, using (A) ΔE* ab (B) ΔE00 and ΔWID (C) metrics, comparing the baseline indexes (T0) with 24 hours (T1), Values below AT indicate adequate acceptability threshold. For ΔE* ab indexes, MTA-Act presented significantly higher values than GP at the interval T1-T0 (*p<0.05). For ΔE00 indexes, MTA-Act presented significantly higher values than all other groups at the interval T1-T0 (*p<0.05) and than MTA-Abo at the interval T3-T0 (^{O}p<0.05). For ΔWID values, positive values indicate whitening, while negative values indicate darkening. Differences within 0-2.5 represents acceptable color changes, while greater values represent perceptible and unaceptable tooth discoloration.

Figure 3. Median and SD values of color differences in samples filled with blood , using (A) ΔE* ab (B) ΔE00 and ΔWID (C) metrics, comparing the baseline indexes (T0) with 24 hours (T1), 30 days (T2), 45 days (T3) and 180 days (T4) after coronal barrier application:. For ΔE* ab and ΔE00 indexes, values below AT indicate adequate acceptability threshold. For ΔWID values, positive values indicate whitening, while negative values indicate darkening. Differences within 0-2.5 represents acceptable color changes, while greater values represent perceptible and unaceptable tooth discoloration.

Figure 4. Median and SD values of color differences in samples filled with blood after dentin bonding agent application, using (A) ΔE* ab (B) ΔE00 and ΔWID (C) metrics, comparing the baseline indexes (T0) with 24 hours (T1), 30 days (T2), 45 days (T3) and 180 days (T4) after coronal barrier application:. For ΔE* ab and ΔE00 indexes, values below AT indicate adequate acceptability threshold. For ΔWID values, positive values indicate whitening, while negative values indicate darkening. Differences within 0-2.5 represents acceptable color changes, while greater values represent perceptible and unaceptable tooth discoloration.

Figure1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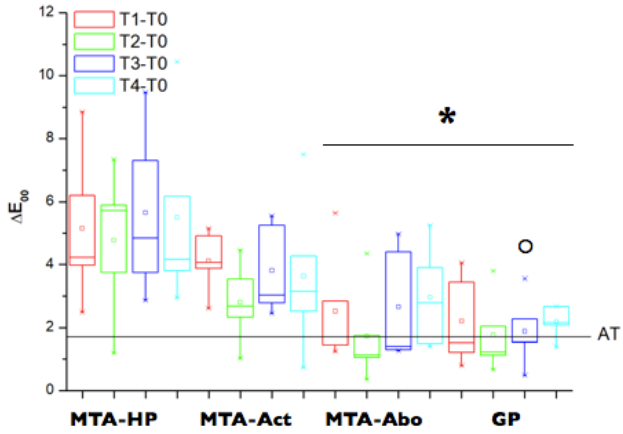
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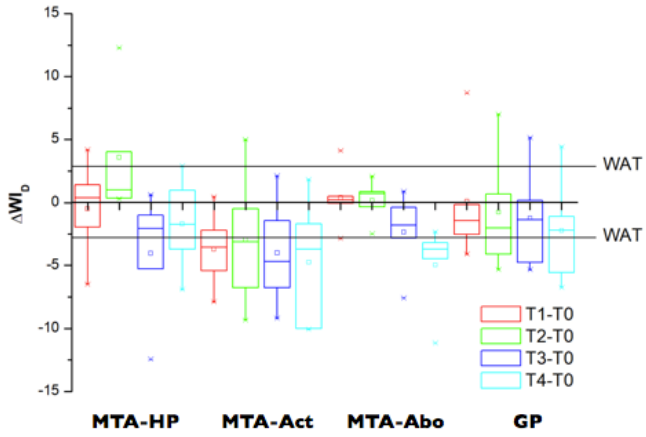
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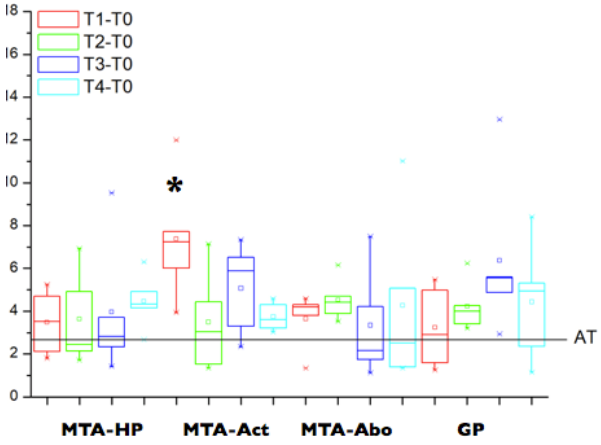
Figure 2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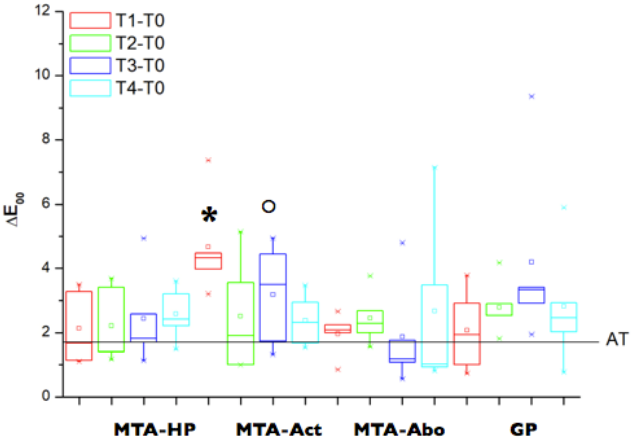
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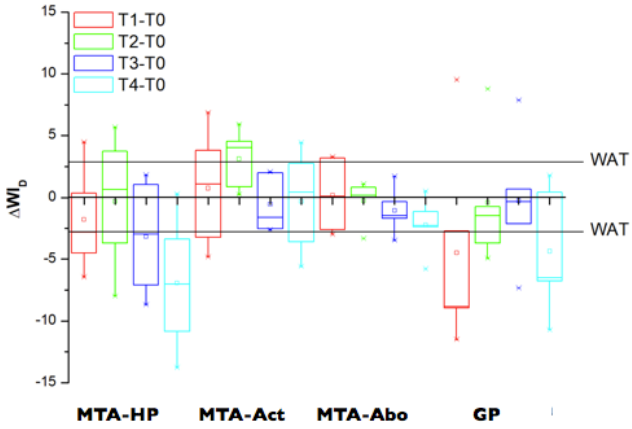
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Figure 3

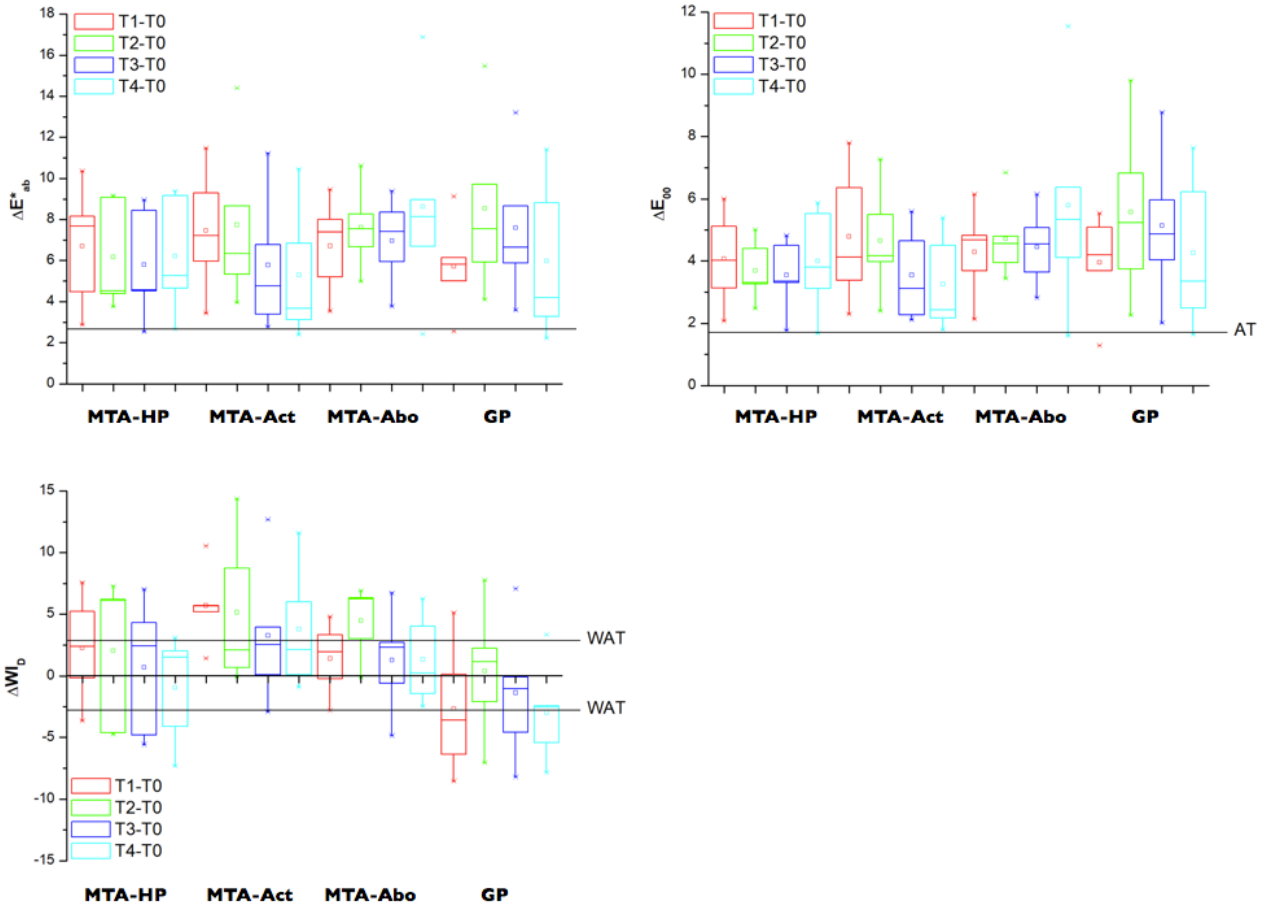
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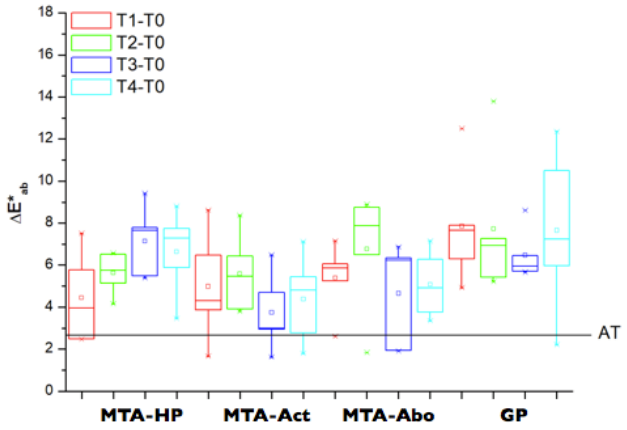
Figure 4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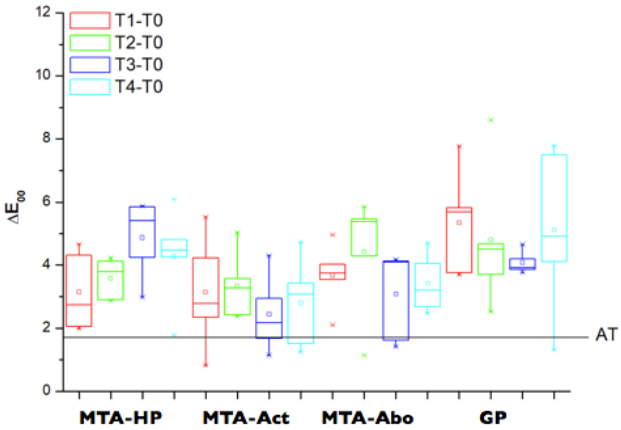
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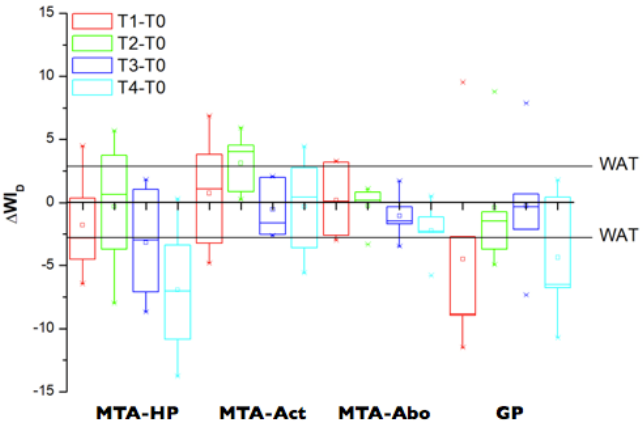
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MTA-Act
MTA-Abo
GP

6.CONSIDERAÇÕES FINAIS

Com base na metodologia empregada e, considerando-se os resultados obtidos e a discussão pertinente, pode-se concluir que:

1.EM RELAÇÃO A SOLUBILIDADE:

- ✓Não houve diferenças significativas de solubilidade entre os materiais ao longo do experimento.
- ✓Os três cimentos à base de silicato apresentaram valores de massa constantes e comportamento adequado de acordo a ISO 6876:2012.

2.EM RELAÇÃO AO TEMPO DE PRESA:

- ✓No tempo de presa inicial os cimentos testados apresentaram semelhança no tempo de presa (20 minutos).
- ✓No tempo de presa final o MTA-A branco apresentou maior tempo em relação aos outros cimentos.

3.EM RELAÇÃO AO pH:

- ✓Todos os cimentos mativeramt pH alcalino longo dos 28 dias.

4.EM RELAÇÃO À LIBERAÇÃO DE ÍONS DE CÁLCIO:

- ✓O cimento BD mostrou aumento da liberação de íons de cálcio mais cedo que os outros materiais, também produzindo níveis mais elevados de liberação de íons na maioria dos períodos experimentais.

5.EM RELAÇÃO À RADIOPACIDADE:

- ✓Todos os cimentos avaliados apresentaram radiopacidade maior que a da dentina, apresentando valores superiores a 3 mm Al (conforme determina a ISO 6876:2012).
- ✓Os cimentos que apresentaram maiores valores de radiopacidade foi MTA Angelus branco, seguido pelo MTA Repair HP.

6. EM RELAÇÃO À PIGMENTAÇÃO DENTÁRIA:

- ✓Todos os cimentos testados promoveram pigmentação coronária.
- ✓Na ausência de sangue, o uso de adesivo dentinario para selar câmara pulpar permitiu que a formulação antiga do MTA Ângelus, contendo óxido de bismuto, proporcionasse alterações de cor aceitáveis ao olho humano.

Finalmente, com base nos resultados desse estudo, observou-se que a formulação nova do MTA-Angelus, contendo tungstato de cálcio, o MTA Repair HP e o Biodentine apresentaram propriedades físico-químicas e radiopacidade adequadas para o uso clínico. Por outro lado, as duas formulações do MTA Ângelus, bem como o MTA Repair HP promoveram pigmentação coronária, sendo necessária cautela quando o emprego desses materiais em uma área estéticamente importante for necessário.

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ANEXO 1

UFRGS - PRÓ-REITORIA DE PESQUISA DA UNIVERSIDADE FEDERAL DO RIO GRANDE DO

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Propriedades físico-químicas e pigmentação coronária causada pelo MTA HP em comparação ao MTA branco convencional.

Pesquisador: Roberta Kochenborger Scarparo

Área Temática:

Versão: 2

CAAE: 97001418.6.0000.5347

Instituição Proponente: Faculdade de Odontologia

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 3.007.591

Apresentação do Projeto:

Trata-se de um projeto de pesquisa de Camila Zimmermann Rabello, do Programa de Pós-Graduação em Odontologia, coordenado pela profa. Roberta Kochenborger Scarparo, do Departamento de Odontologia Conservadora da Faculdade de Odontologia (FO) da UFRGS. O estudo objetiva avaliar as propriedades físico-químicas do cimento MTA-HP, empregado em obturações dentárias, avaliando parâmetros tais como solubilidade, tempo de presa, pH e liberação de íons cálcio, além de avaliar pigmentação coronária causada pelo cimento MTA HP (Angelus,Londrina, Brasil). Em comparação ao MTA branco convencional, produzido pela mesma empresa e já amplamente empregado em diversos procedimentos. Para esse último objetivo, serão utilizados dentes extraídos de pacientes. Os autores descrevem que os pacientes irão assinar um termo de consentimento de doação dos dentes na presença de duas testemunhas e que serão convidados a participar da pesquisa pacientes que procurarem a disciplina de Exodontia da FO da UFRGS, e que terão os dentes extraídos, conforme a indicação do plano de tratamento e registrado em prontuário. Está previsto o uso de 120 dentes. A análise da capacidade do MTA HP e do MTA Branco em alterar a coloração dentária será avaliada em um modelo com dentes humanos extraídos, no qual esses materiais serão colocados em contato com a dentina na região cervical dos dentes, simulando o seu emprego clínico como material de selamento cervical. Tal propriedade dos materiais será avaliada tanto quando em contato com sangue quanto em contato com solução salina. Além disso, o emprego de barreira de adesivo dentinário subjacente aos

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