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DESCOLORAÇÃO DENTÁRIA INDUZIDA POR MATERIAIS UTILIZADOS NA TERAPIA REGENERATIVA ENDODÔNTICA E A EFETIVIDADE DO CLAREAMENTO INTRA-CORONÁRIO

Aracaju

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Orientador: Prof. Dr. André Luís Faria e Silva

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RESUMO

Este estudo avaliou a descoloração coronária induzida por materiais utilizados na terapia regenerativa endodôntica bem como o efeito do clareamento interno na descoloração. Trinta terceiros molares foram alocados aleatoriamente de acordo com a etiologia da descoloração (n = 10): MTA - agregado trióxido mineral branco, TAP - pasta tri-antibiótica e BLD - sangue bovino (controle). Após a medição da cor inicial do dente, os canais radiculares foram parcialmente preenchidos com MTA, TAP, ou uma bolinha de algodão com sangue bovino foi colocada na câmara pulpar. As alterações de cor foram avaliadas após 30, 60 e 180 dias usando um espectrofotômetro portátil (sistema CieLab). Após a descoloração, o clareamento interno dos dentes foi realizado com uma mistura de perborato de sódio e peróxido de hidrogênio a 20% inserido na câmara pulpar e substituído semanalmente por 21 dias. A cor da coroa foi medida antes de cada substituição do agente clareador e uma semana após a última. No início, após a coloração e clareamento, os dados de cada parâmetro de cor foram analisados individualmente por ANOVA de uma via, enquanto as diferenças em ΔE foram avaliadas por ANOVA de 2 vias medidas repetidas ($\alpha = 0,05$). Após o tempo de coloração, TAP resultou em amostras mais escuras e mais verdes, e não foi observada diferença entre os dentes corados com BLD ou MTA. O clareamento interno resultou na melhora da cor dos dentes sem diferença na mudança de cor entre os agentes etiológicos. No entanto, as amostras coradas com TPA ficaram mais escuras, mais verdes e azuis após o término dos procedimentos de clareamento. Em conclusão, o TPA resultou em descoloração dentária mais significativa e a pior cor permaneceu após os procedimentos de clareamento dentário.

Palavras-chave: Agregado de trióxido mineral; Pasta tri-antibiótica; Clareamento dentário; Descoloração dentária.

ABSTRACT

This study evaluated the coronal discoloration induced by materials used in endodontic regenerative therapy, as well as the effect of internal bleaching on discoloration. Thirty third molars were randomly allocated according to discoloration etiology (n = 10): MTA – White Mineral Trioxide Aggregate, TAP - triple antibiotic paste and BLD - bovine blood (control). After tooth color measurement at baseline, the root canals were partially filled with MTA, TAP, or a cotton pellet with bovine blood was placed into the pulpal chamber. The color changes were assessed after 30, 60 and 180 days using a portable spectrophotometer (CieLab system). Following the discoloration, internal tooth bleaching was performed with a mixture of sodium perborate and hydrogen peroxide inserted into de pulpal chamber and replaced weekly for 21 days. The crown color was measured before each replacement of bleaching agent and one-week after the last one. At baseline, after staining and bleaching, data of each color parameter were individually analyzed by one-way ANOVA, while differences on ΔE were assessed by 2-way repeated measures ANOVA ($\alpha = 0.05$). After the staining time, TAP resulted in darker and greener specimens, and no difference was observed between teeth stained with blood or MTA. Walking bleaching resulted in improved tooth color without difference on color change among the etiologic agents. However, specimens stained with TPA remained darker, greener and bluer after the end of bleaching procedures. In conclusion, TPA resulted in more significant tooth discoloration and the worst color remained after tooth bleaching procedures.

Key-Words: Mineral Trioxide Aggregate; Triple antibiotic paste; Tooth bleaching; Tooth discoloration.

SUMÁRIO

1-INTRODUÇÃO

A descoloração dentária é considerada como um problema estético e frequentemente é resultado de traumatismo, remoção inadequada do tecido da câmara pulpar ou uso de materiais endodônticos por muito tempo (1). A ruptura dos vasos sanguíneos e consequente hemorragia na câmara de pulpar por trauma grave pode causar uma descoloração coronária pela penetração de componentes sanguíneos dentro dos túbulos dentinários (2). Por isso, os subprodutos da degradação do sangue, tais como hemossiderina, hemina, hematina e hematoidina, são liberados para os túbulos dentinários e produzem alterações cromáticas dentárias (3). Mecanismo semelhante é verificado quando remanescentes de polpa são deixados dentro da câmara pulpar como consequência de uma cavidade de acesso inadequada para o tratamento endodôntico (4). Além disso, outras causas iatrogênicas da descoloração dos dentes durante a terapia endodôntica podem ser associadas a alguns materiais colocados na câmara pulpar ou no canal radicular, incluindo cimentos e medicamentos intracanais (5). Assim, a descoloração dos dentes pode ser observada durante tratamento endodôntico convencional ou pela terapia endodôntica regenerativa.

A terapia endodôntica regenerativa é uma alternativa para tratar canais radiculares infectados em dentes com rizogênese incompleta visando o alongamento radicular e espessamento das paredes dentinárias(6). A terapia consiste na desinfecção dos canais radiculares, seguida da introdução de um coágulo sanguíneo e / ou células tronco / progenitoras no espaço do canal radicular (7). Uma vez que o sangue é intencionalmente introduzido no espaço pulpar, é razoável esperar que qualquer grau de descoloração da coroa e preocupações estéticas tenha sido descritas para essa técnica. Além disso, a pasta tri-antibiótica utilizada para desinfetar o canal radicular apresenta minociclina, que é capaz de

induzir a descoloração dos dentes (8). Outro material endodôntico utilizado durante a terapia regenerativa como barreira intracoronária é o agregado de trióxido mineral (MTA) devido suas vantagens de capacidade de selamento adequada, biocompatibilidade e atividades antibacterianas (9). No entanto, demonstrou-se que o MTA branco também é capaz de causar descoloração da coroa (9). Portanto, apesar do aumento do risco de descoloração dentária causada pela terapia endodôntica regenerativa, pouca informação está disponível sobre a eficácia de procedimentos de clareamento no tratamento de dentes escurecidos após esta terapia(10).

Independentemente do fator etiológico, a abordagem mais conservadora para tratar o dente escurecido não vital é pelo clareamento interno, enquanto a técnica de *walking bleaching* é amplamente utilizada para esse fim (2,11). Esta técnica é realizada por colocação do agente clareador na câmara pulpar seguida de restauração provisória da cavidade de acesso. Após alguns dias, o efeito de clareamento é avaliado e, se necessário, o agente de clareamento é substituído (2,12). Vários agentes de clareamento são utilizados na técnica *walking bleaching*, incluindo perborato de sódio, peróxido de carbamida ou peróxido de hidrogênio; enquanto a associação entre peróxido de hidrogênio e perborato de sódio apresenta altas taxas de sucesso (2). Apesar das diferenças de apresentação, segurança e eficácia, todos esses agentes de clareamento produzem peróxido de hidrogênio que interage com o componente orgânico do tecido dentinário resultando em efeito de clareamento (12).

2-OBJETIVO

Avaliar o grau de alteração de cor das coroas dentárias causada pelo sangue ou materiais utilizados durante a terapia regenerativa; bem como o efeito do clareamento interno utilizando a técnica de *walking bleaching*.

Objetivos específicos

1. Mensurar a mudança de cor de coroa dentária causada pela colocação agregado trióxido mineral ou pasta triantibiótica na entrada dos canais radiculares; ou de sangue bovino na câmara pulpar;
2. Avaliar a efetividade do clareamento interno (*walking bleaching*) em função da alteração de cor causada por diferentes agentes etiológicos.

3-METODOLOGIA

3.1-Delineamento experimental

Este estudo *in vitro* avaliou o efeito do agente etiológico na alteração de cor da coroa dentária em três níveis (sangue, MTA branco, e pasta tri-antibiótica) nas mudanças de cor do dente e na eficácia da técnica de clareamento dentário interno. As alterações de cor foram avaliadas usando um espectrofotômetro com o sistema CIELab após 30, 60 e 180 dias de procedimentos de escurecimento; e semanalmente durante os procedimentos de clareamento interno durante 21 dias. O protocolo do estudo foi aprovado pelo Comitê de Ética em Pesquisa envolvendo seres humanos da Universidade Federal de Sergipe (CAAE: 55902316.4.0000.5546).

3.2-Cálculo da amostra

O cálculo do tamanho da amostra foi realizado considerando o resultado principal como ΔE00. O cálculo foi baseado em desvio padrão médio observado em um estudo piloto (1.20), uma diferença mínima detectável em 1,77 (limite de aceitabilidade) (13), poder de teste 0,80 e $\alpha = 0,05$, para três grupos experimentais a serem submetidos a um teste de ANOVA de uma via. O cálculo foi realizado utilizando o software estatístico SigmaStat v.3.5 (Systat Software Inc., Chicago, IL, EUA) e o tamanho da amostra requerida foi determinado como $n = 10$.

3.3-Preparo das amostras

Trinta terceiros molares hígidos extraídos de humanos, sem alteração significativa de cor e trincas, que ficaram armazenados em um recipiente com água destilada, foram selecionados para este estudo. A abertura coronária para acesso aos canais radiculares foi

realizado com pontas diamantadas (número 1013; KG Sorensen, Barueri, SP, Brasil), para acesso, seguido de remoção do teto pulpar e refinamento das paredes circundantes com brocas endo-Z (Dentsply Maillefer, Ballaigues, Switzerland). Após remoção de todo o tecido pulpar com limas de fino calibre número 10 e 15 (Dentsply Maillefer, Ballaigues, Switzerland) , os canais foram alargados com brocas Gattes-Glidden (número 2, Dentsply Maillefer, Ballaigues, Switzerland) utilizando um stop de silicone como delimitador para obtermos o 5 mm de profundidade. A *smear layer* produzida foi removida com irrigação de com hipoclorito de sódio a 2,5% seguido por EDTA a 17%, com irrigação final com água destilada. Uma porção de Coltosol (Vigodent, Rio de Janeiro, RJ, Brasil) foi inserida dentro dos canais e condensada com o condensador tipo Paiva até a parte mais apical, de forma a manter apenas 3 mm de abertura do canal desobstruídos. O preparo das amostras está ilustrado na Figura 1, e estas foram aleatoriamente distribuídas entre as condições experimentais através de lista randômica.

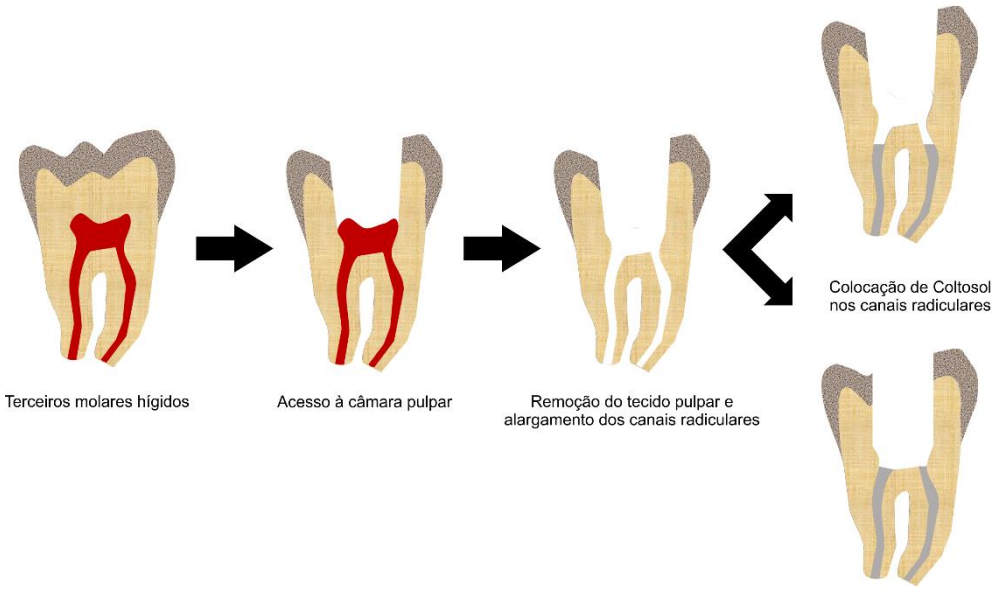

Figura 1. Preparo das amostras para a colocação dos materiais na entrada dos canais radiculares, ou sangue na câmara pulpar.

3.4-Avaliação inicial de cor

A cor das amostras na avaliação inicial foi medida com um espectrofotômetro (SP60, X-Rite, Grand Rapids, MI, EUA), no modo de reflectância, usando o sistema do *Commission Internationale de l'Eclairage* - CIELab (L *: branco/ preto; a *: vermelho/ verde; b *: amarelo/ azul) – Figura 2. A leitura da cor foi realizada nas coroas dos molares tanto da face vestibular quanto lingual, com as amostras posicionados sobre um fundo branco (L * = 95.2, a * = -1.2, b * = 0.3). Foram realizadas três medidas por face, e a média das seis leituras foi registrada.

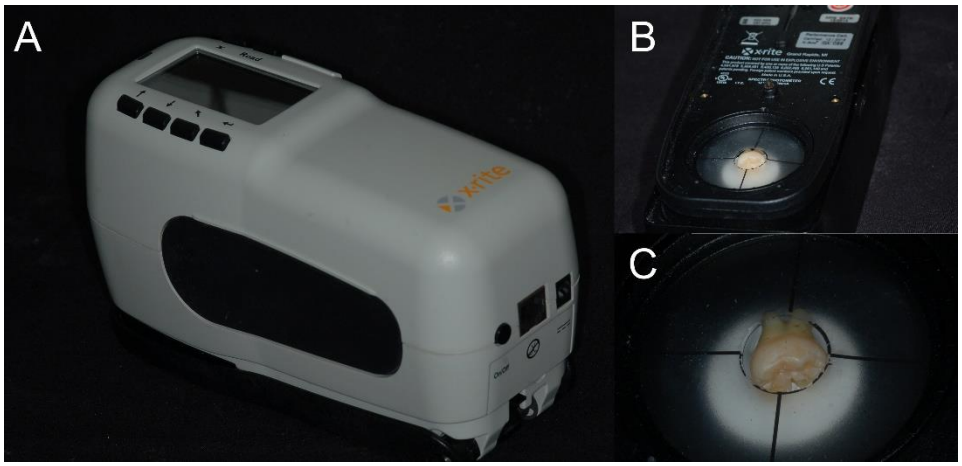

Figura 2. (A) – Espectrofotômetro portátil SP60, (B) coroa dental posicionada no porta-amostras do aparelho, e (C) imagem aproximada destacando a leitura sobre o fundo branco.

3.5 - Colocação do material para descoloração

No espaço vazio dos 3 mm cervicais dos canais radiculares, foi inserido MTA branco (MTA Angelus, Londrina, PR, Brasil) com a espáulta de inserção e condensado com o condensador tipo Paiva número 1 ou uma pasta tri-antibiótica composta por ciprofloxacina, minociclina e metronidazol tendo como veículo Propilenoglicol (n =10), com auxílio de lentulo. Em seguida, uma bolinha de algodão umedecida em água destilada foi colocada na entrada do canal e a câmara pulpar foi preenchida com Coltosol. No grupo controle, um algodão embebido em sangue bovino foi introduzido na câmara pulpar, que foi

selada com Coltosol – Figura 3. Todas as amostras foram armazenadas em água destilada em estufa bacteriológica a 37˚C, sendo a cor da coroa foi avaliada após 30, 60 e 180 dias, da mesma forma descrita anteriormente.

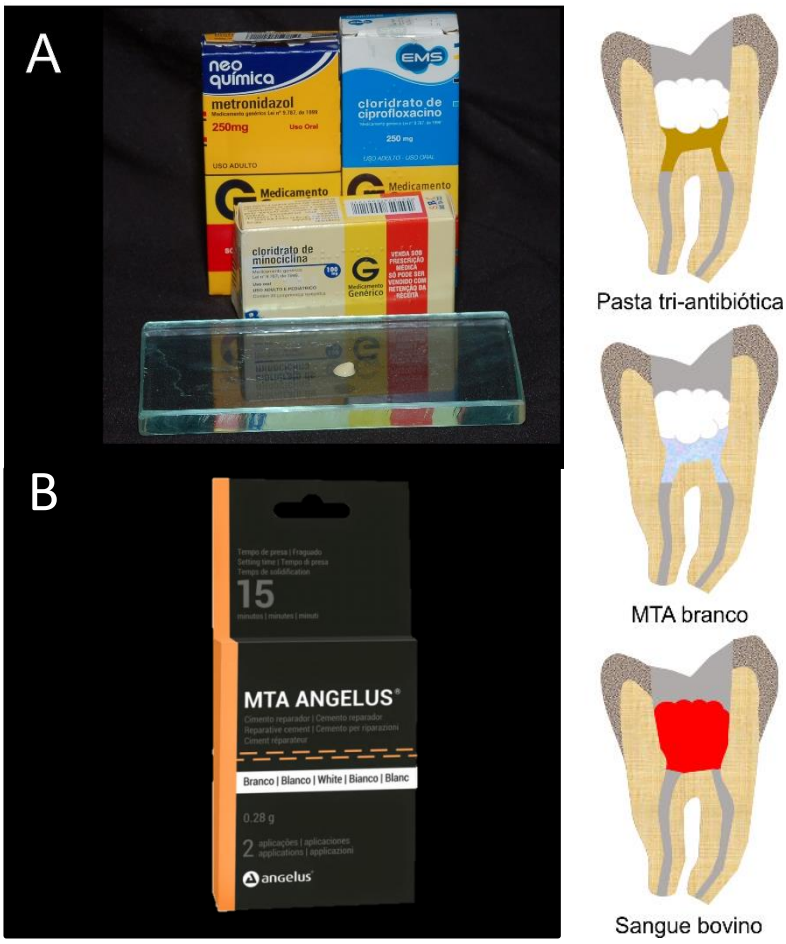

Figura 3. Figura ilustrativa da colocação dos materiais e sangue nas amostras. Laranja – pasta tri-antibiótica, cinza claro – MTA branco, vermelho – bolinha de algodão com sangue. (A) antibióticos utilizados para preparo da pasta, e (B) MTA branco utilizado no estudo.

3.6-Clareamento dental

Após a conclusão dos procedimentos de descoloração, as restaurações provisórias e as bolinhas de algodão foram removidas e as cavidades foram limpas com água destilada. Nas amostras onde o MTA ou a pasta tri-antibiótica foram utilizadas, estes materiais foram removidos e substituídos por Coltosol, mantendo a câmara pulpar sem qualquer material. O procedimento de clareamento interno foi realizado utilizando um agente clareador a base de

perborato de sódio e peróxido de hidrogênio a 20% (Whiteness Perborato, FGM, Joinville, SC, Brasil) – Figura 4. O agente clareador foi misturado conforme recomendações do fabricante e a pasta resultante colocada na câmara pulpar, que foi então selada com Coltosol. As amostras foram armazenadas em água destilada a 37 ° C por uma semana. Após este período, a cor foi mensurada novamente e o agente clareador renovado. As amostras foram clareadas durante três semanas e a cor foi avaliada semanalmente.

Figura 4. Agente clareador utilizado na técnica *walking-bleaching*.

3.7-Análise dos dados

Análise de Variância (ANOVA) de uma via foi utilizada para verificar possíveis diferenças nos parâmetros de cor, mensurados antes dos procedimentos de colocação dos materiais ou sangue, entre as amostras alocadas para cada condição experimental. Comparações entre os agentes etiológicos também foram realizadas após o final do período de descoloração e de clareamento usando ANOVA de uma via. A mudança global de cor (ΔE_{00})

durante o período de descoloração foi calculada baseado em fórmula descrita em estudo prévio anterior (14) para cada tempo de avaliação baseado nos valores mensurados antes do início da descoloração. Já para as mudanças de cor causadas pelo clareamento, ΔE_{00} foi calculado para cada tempo de avaliação baseado nos dados observados após 6 meses de descoloração. Dados de ΔE_{00} foram individualmente analisados por ANOVA de duas vias com medidas repetidas, sendo que o "tempo de avaliação" foi definido como fator de repetição. Comparações múltiplas foram realizadas com teste de Tukey e o nível de significância foi definido em 95% para todas as análises.

4-RESULTADOS

Tooth discoloration induced by regenerative endodontic materials and the effectiveness of walking bleaching

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Abstract

This study evaluated the coronal discoloration induced by different materials, or blood as well as the effect of internal bleaching on discoloration. After tooth color measurement at baseline, the root canals of molars were partially filled with white mineral trioxide aggregate (MTA), triple antibiotic paste (TAP), or a cotton pellet with bovine blood (control) was placed into the pulp chamber. The color changes were assessed at 30, 60, and 180 days after the procedure. Following the discoloration, internal bleaching was performed with a mixture of sodium perborate and hydrogen peroxide inserted into the pulp chamber, and this mixture was replaced weekly for 3 weeks. The crown color was measured before each replacement of bleaching agent and 1 week after the last one. At baseline, after staining, and after bleaching, the data of each color parameter were individually analyzed by one-way ANOVA, while differences in pooled color changes (ΔE_{00}) were assessed by two-way repeated measures ANOVA ($\alpha = 0.05$). After the staining period, the TAP-stained specimens were darker and greener than the other specimens, and no difference was observed between the teeth stained with blood or MTA. The walking bleach technique resulted in an improved tooth color without a difference in the color changes among the different groups. However, the specimens stained with TAP remained darker, greener, and bluer after the end of the bleaching procedures. In conclusion, the TAP-stained specimens had the greatest tooth discoloration, and the discoloration remained the highest after the walking bleach technique.

Keywords: Dental pulp cavity; Esthetics, dental; Root canal filling materials; Root canal therapy; Tooth bleaching.

Introduction

Tooth discoloration is considered as an aesthetic problem that frequently results from trauma, incomplete removal of pulp tissue, or the use of some endodontic materials for long periods of time.[1] The rupture of blood vessels and a consequent hemorrhage in the pulp chamber due to severe trauma can cause coronal discoloration by the penetration of blood components within the dentinal tubules.[2] Hence, subproducts from the degradation of blood, such as hemosiderin, hemine, hematin, and hematoidin, are released into the dentinal tubules and yield tooth chromatic alterations.[3] Furthermore, other causes of tooth discoloration during endodontic therapy are related to some materials placed in the pulp chamber or root canal, including sealers and intracanal medications used in conventional endodontic procedures as well as in regenerative endodontic therapy.[4,5] Thus, tooth discoloration can be observed after regenerative endodontic procedures.

Regenerative endodontic therapy is indicated to treat infected root canals in immature teeth, aiming to induce root elongation and/or maturation.[6] The therapy consists of root canal disinfection followed by introduction of a blood clot and/or stem/progenitor cells into the root canal space.[7] Since blood is intentionally introduced into the pulp space, it is reasonable to expect some degree of crown discoloration, and esthetic concerns have been described for this technique. In addition, the triple antibiotic paste (TAP) used to disinfect the root canal contains minocycline, which is known to induce tooth discoloration.[8] Another endodontic material used as an intracoronal barrier during regenerative therapy is white mineral trioxide aggregate (MTA); this material has many advantages, including a high-quality sealing ability, biocompatibility, and antibacterial activity.[9] However, it has been demonstrated that MTA also causes crown discoloration.[9] Despite the increased risk of tooth discoloration caused by regenerative endodontic therapy, few studies are available regarding the efficacy of bleaching procedures on discolored teeth.[10]

The most conservative approach to treat a nonvital discolored tooth is by internal bleaching; the walking bleach technique is largely used for this purpose.[2,11] This technique is performed by placement of a bleaching agent into the pulp chamber, followed by provisional restoration of the access cavity. After a few days, the bleaching effect is evaluated, and, if necessary, the bleaching agent is replaced.[2,12] Several bleaching agents have been used in the walking bleach technique, including sodium perborate, carbamide peroxide, and hydrogen peroxide; meanwhile, the combination of hydrogen peroxide with sodium perborate has demonstrated high success rates.[2] Despite differences regarding the presentation, safety, and

effectiveness, all of these bleaching agents yield hydrogen peroxide, which oxidizes the organic components of dentinal tissue, causing a bleaching effect.[12] Thus, the aims of this study were to evaluate the tooth discoloration caused by blood or endodontic materials used during regenerative therapy as well as to assess the bleaching effect achieved with the walking bleach technique. We hypothesized that there would be no difference in the color changes caused by blood/regenerative endodontic materials or on the ultimate color following tooth bleaching.

Methodology

Experimental design

This single-factor study evaluated the effect of three staining agents (blood, white MTA, and TAP) on tooth color changes and the effectiveness of the walking bleach technique. Color changes were assessed by using a spectrophotometer with the CIE L* a* b* system following staining periods of 30, 60, and 180 days as well as weekly (for 3 weeks) after the walking bleach procedure. This study was approved by the Research Ethics Committee of the local University of (CAAE: 55902316.4.0000.5546).

Sample size

Sample size calculation was carried out considering the main outcome as ΔE00. The calculation was based average standard deviation observed in a pilot study (1.20), a minimum detectable difference in means of 1.77 (acceptability threshold),[13] power test of 0.80, and $\alpha = 0.05$, for three experimental groups to be submitted to one-way ANOVA. The calculation was performed using the statistical software SigmaStat v.3.5 (Systat Software Inc., Chicago, IL, USA) and required sample size was determined to be n=10.

Specimen preparation

Thirty sound third molars, without any significant coronal color alteration or cracks, were selected for this study. Coronal access was performed using a #1013 round diamond bur (KG Sorensen, Barueri, SP, Brazil) followed by an Endo-Z bur (Dentsply Sirona, Ballaigues, Switzerland). Afterwards, the pulp tissue was removed and the root canals were enlarged with a #2 Gates-Glidden drill (Dentsply Sirona, Ballaigues, Switzerland) at 5 mm deep. The smear layer was removed with 2.5% sodium hypochlorite, followed by 17% EDTA, and final rinsing with distilled water. Coltosol (Vigodent, Rio de Janeiro, RJ, Brazil) was placed inside

the root canal space and vertically condensed up to the most apical portion of the root. Then, a 3-mm portion of the cervical entrance of the root canal was unobstructed.

Baseline measurements

The color of the specimens at baseline was measured with a spectrophotometer (SP60, X-Rite, Grand Rapids, MI, USA) in reflectance mode, using the CIE L*a*b* system (L*: white/black; a*: red/green; b*: yellow/blue). The color parameters were measured at the buccal and lingual faces, with the specimens positioned over a white background (L* = 95.2, a* = -1.2, b* = 0.3). Three measurements were performed per specimen, and the average was recorded.

Staining procedures

White MTA (Angelus, Londrina, PR, Brazil), TAP (composed of ciprofloxacin, minocycline, and metronidazole), or propilenoglicol as the vehicle (n =10) was placed in the empty space of the cervical portion (3 mm) of the root canal. A moist cotton pellet was placed in the canal's entrance, and the pulp chamber was sealed with Coltosol. In the control group, a cotton pellet soaked with bovine blood was placed in the pulp chamber, which was then sealed with Coltosol. The specimen preparation and procedures of the staining agent placements are illustrated in Figure 1. The specimens were stored at 37 °C in distilled water during the entire storage time. The color was measured after storage for 30, 60, and 180 days using the same protocol described at baseline.

Walking bleach procedures

After the staining procedures were complete, the provisional restorations and cotton pellets were removed, and the cavities were cleaned with distilled water. For specimens treated with MTA or TAP, these materials were removed and replaced by Coltosol, maintaining the pulp chamber without any material. The walking bleach procedure was performed using a sodium perborate/hydrogen peroxide agent (Whiteness Perborate, FGM, Joinville, SC, Brazil). The bleaching agent was mixed, and the resulting paste was placed into the pulp chamber, which was then sealed with Coltosol. The specimens were stored in distilled water at 37 °C for 1 week. After this period, the color was measured again, and the bleaching agent was renewed. The specimens were bleached for 3 weeks, and the color was assessed weekly.

Statistical analyses

Data analysis was performed using the SigmaStat v.3.5 statistical software package (Systat Software Inc., Chicago, IL, USA). Possible differences in color parameters at baseline among the specimens allocated to each etiologic agent were assessed by one-way analysis of variance (ANOVA). Comparisons among the etiologic agents for each color parameter were also analyzed after the staining period ended and after the last bleaching procedure using one-way ANOVA. The pooled color changes (ΔE_{00}) during the staining period were calculated using the formula described previously,[14] based on data from baseline. Color changes caused by the tooth-bleaching procedures were calculated based on the color measured after staining for 6 months using the same formula. Data of ΔE_{00} were individually analyzed by two-way repeated measures ANOVA, while the "time assessment" was used as a repetition factor. Multiple comparisons were performed by the post-hoc Tukey's test, and the significance level was set at 95% for all data analyses.

Results

Table 1 compares the results of the various etiologic agents for each color parameter measured at baseline. No difference was observed among the specimens allocated to receive the different staining protocols, irrespective of the parameter evaluated.

The results of the color changes caused by the staining procedures are displayed in Table 2. For the pooled color change (ΔE_{00}), two-way repeated measures ANOVA showed that both the "assessment time" ($p = 0.004$) and "etiologic agent" ($p < 0.001$) affected the results and that the interaction between the factors was significant ($p < 0.001$). After 1 and 2 months, the TAP induced the highest color change values, and no difference was observed between the other agents. On the other hand, MTA presented a greater ΔE_{00} than TAP, and blood resulted in intermediate values. A greater color change was observed for MTA and blood after 6 months, while ΔE_{00} for TAP was reduced at that time when compared to the values observed after 2 months. After the entire staining period, differences among the etiologic agents were observed for the parameters L* ($p < 0.001$) and a* ($p < 0.001$), and specimens stained with TAP presented the lowest values of L* and a*. However, all three etiologic agents presented similar values of b* ($p = 0.65$) after staining for 6 months.

Two-way repeated measures ANOVA showed that only the "assessment time" ($p < 0.001$) affected the color change (ΔE_{00}) during the walking bleach procedure – Table 3. The etiologic agent ($p = 0.072$, $p = 0.379$ for interaction) did not affect the effectiveness of the bleaching procedure. As expected, treatment using the walking bleach technique for

additional weeks resulted in a further bleaching effect. At the end of the walking bleach procedure, one-way ANOVA determined that the "etiologic agent" caused a significant effect ($p = 0.001$, for all color parameters). Irrespective of the color parameter evaluated, the lowest values were observed for specimens stained with TAP, while MTA and blood presented similar color changes. The color parameter behaviors during the entire experiment are described in Figure 2. To facilitate the visualization of color changes, the values of L*, a*, and b* were converted to an RGB (red, green, and blue) system, and colored squares were drawn using the software CorelDraw Graphics Suite X8 (Corel Corporation, Ottawa, ON, Canada) in the RGB model – Figure 3.

Discussion

Tooth discoloration caused by blood or endodontic materials used during regenerative therapy can result in esthetic concerns for patients when they occur in anterior teeth. Among the treatments available, tooth bleaching procedures are a conservative approach to solve tooth discoloration, but their effectiveness is strongly dependent on the etiology of the discoloration.[2] In the present study, the placement of MTA into the root canals resulted in a higher global color change after 6 months, and the lowest ΔE_{00} was observed for TAP-stained teeth. However, teeth stained with TAP were darker and greener than those stained with blood or MTA. Moreover, despite the fact that similar color changes were achieved with the walking bleach procedure for 3 weeks, teeth stained with TAP remained darker and greener, and these specimens presented an ultimate color closer to blue. Therefore, the hypothesis of this study was rejected.

TAP is composed of three antibiotics, including minocycline, which is a semi-synthetic antibiotic derived from tetracycline[15] Unlike tetracycline, which can bind to dentin tissue through chelation to calcium, a subproduct of minocycline breakdown called hemosiderin is able to chelate iron ions to form an insoluble complex with the tooth.[16,17] Therefore, minocycline can induce tooth discoloration in those of any age and by direct contact with tooth tissue. Following its exposure to either oxygen or bacterial activity, minocycline becomes darker (i.e., turns black) due to an oxidation reaction.[16,18] In the present study, an increase of yellowness was observed in specimens stained with TAP, since this paste presents that type of coloration during its insertion into the root canal. However, this yellowness was reduced following the time of exposure to TAP, and this color change is explained by the oxidation of minocycline. Another important observation was that the increased discoloration observed for TAP-stained teeth after a 2-month staining period was

strongly affected by a lower lightness (L*) value and a higher greenness (a*) value. In fact, a specific standard of tooth discoloration with a singular occurrence of “black or green” aspects have been reported for permanent teeth stained with minocycline.[19]

Interestingly, the lightness of specimens increased substantially for all etiologic agents after storage for 6 months, while a significant reduction in yellowness was observed. We hypothesized that the etiologic agents were gradually solubilized due to storage in water. Considering that the pulp chamber was empty (except for the presence of a cotton pellet for the blood-stained teeth), removing the filling materials from this space resulted in a higher translucency of specimens and visualization of a white background. As a result, whiter (increased L* value) and bluer (reduced b* value) specimens were observed. Since TAP-stained specimens presented a greater reduction of L* after 2 months, increasing the lightness reduced the color changes. Furthermore, the lowest reduction of b* after 6 months for TAP-stained specimens resulted in the lowest ΔE_{00} among the etiologic agents. However, the lowest L* and a* values remained for TAP-stained specimens during the entire experiment, and the darkest and greenest teeth were observed after the staining period.

The highest global color change during the staining period was observed for MTA, which is a bioactive silicate cement.[20,21] In fact, a slight color change was observed for MTA-stained specimens stored for 2 months, and the color measured after 6 months was strongly affected, as seen by increased lightness values and reduced yellowness values. Among the soluble components of MTA, calcium silicate is leached out from the material over a five-week period.[22] The ability of MTA to darken the tooth structure has been related to the presence of radiopacifying agents such as bismuth oxide.[23] However, alternative radiopacifying agents such as zirconium oxide and tantalum oxide have been introduced into the composition of MTA to reduce the risk to tooth discoloration.[24] A similar behavior was observed for the specimens containing a cotton pellet soaked with blood and placed into the pulp chamber. The mechanism explaining the possible staining caused by blood is related to the accumulation of hemoglobin molecules or other hematin molecules.[25] The hemolysis of these molecules releases heme groups, which can cause darkening of the tooth structure as they produce black iron sulfide.[26] Therefore, a reduction of lightness values and an increase of redness values following blood exposure to the specimens could be expected. However, the results of the present study did not demonstrate a significant color change caused by the placement of a blood-soaked cotton pellet into the pulp chamber.

In regard to the tooth bleaching, it has been demonstrated that darker teeth are more

prone to bleaching,[27] but similar color changes were observed among the etiologic agents under the assessed bleaching procedures. The bleaching procedure was performed by the placement of paste containing sodium perborate and 20% hydrogen peroxide into the pulp chamber. Sodium perborate breaks down to produce sodium metaborate, hydrogen peroxide, and oxygen when exposed to acid, warm air, and water.[2] Hydrogen peroxide is well known as a strong oxidizing agent when it reacts with other substances as it can release hydrogen peroxide anions and reactive oxygen.[28] Hence, the tooth-bleaching effect is achieved by the oxidation of organic structures from enamel and dentin.[29] As expected, the walking bleach procedures resulted in significant improvement of tooth color, despite the low peroxide concentration used, since dentin tissue with a high organic content was stained by the protocols used in the present study. Similar results have been observed in a prior study using 37% carbamide peroxide.[10] Even though the bleaching procedures significantly improved the tooth color, the ultimate color of the specimens stained with TAP remained darker, greener, and bluer than those stained with the other etiologic agents. However, it is possible that extending the walking bleach procedure for additional weeks would result in a similar ultimate color, since the bleaching effect is limited in whiter teeth.[26]

Conclusions

The findings of the present study demonstrated that increased tooth discoloration occurred after treatment with the triple antibiotic paste, and this color might hinder a satisfactory tooth color from being obtained with the walking bleach technique.

References

1- Lenherr P, Allgayer N, Weiger R, Filippi A, Attin T, Krastl G. Tooth discoloration induced by endodontic materials: a laboratory study. Int Endod J. 2012 Oct;45(10):942-9. https://doi.org/10.1111/j.1365-2591.2012.02053.x.

2- Plotino G, Buono L, Grande NM, Pameijer CH, Somma F. Nonvital tooth bleaching: a review of the literature and clinical procedures. J Endod. 2008 Apr;34(4):394-407. https://doi.org/10.1016/j.joen.2007.12.020.

3- Arens D. The role of bleaching in esthetics. Dent Clin North Am. 1989 Apr;33(2):319-36.

4- Krastl G, Allgayer N, Lenherr P, Filippi A, Taneja P, Weiger R. Tooth discoloration induced by endodontic materials: a literature review. Dent Traumatol. 2013 Feb;29(1):2-7. https://doi.org/10.1111/j.1600-9657.2012.01141.x.

5- Kahler B, Rossi-Fedele G. A review of tooth discoloration after regenerative endodontic therapy. J Endod. 2016 Apr;42(4):563-9. https://doi.org/10.1016/j.joen.2015.12.022.

6- Wingler R, Kaufman AY, Lin S, Steinbock N, Hazan-Molina H, Torneck CD. Revascularization: a treatment for permanent teeth with necrotic pulp and incomplete root development. J Endod. 2013 Mar;39(3):319-26. https://doi.org/10.1016/j.joen.2012.11.014.

7- Cao Y, Song M, Kim E, Shon W, Chugal N, Bogen G, Lin L, Kim RH, Park NH, Kang MK. Pulp-dentin regeneration: current state and future prospects. J Dent Res. 2015 Nov;94(11):1544-51. https://doi.org/10.1177/0022034515601658.

8- Kim JH, Kim Y, Shin SJ, Park JW, Jung IY. Tooth discoloration of immature permanent incisor associated with triple antibiotic therapy: a case report. J Endod. 2010 Jun;36(6):1086-91. https://doi.org/10.1016/j.joen.2010.03.031.

9- Marconyak LJ Jr, Kirkpatrick TC, Roberts HW, Roberts MD, Aparicio A, Himel VT, Sabey KA. A Comparison of coronal tooth discoloration elicited by various endodontic reparative materials. J Endod. 2016 Mar;42(3):470-3. https://doi.org/10.1016/j.joen.2015.10.013.

10- Santos LG, Felippe WT, Souza BD, Konrath AC, Cordeiro MM, Felippe MC. Crown discoloration promoted by materials used in regenerative endodontic procedures and effect of dental bleaching: spectrophotometric analysis. J Appl Oral Sci. 2017 Mar-Apr;25(2):234-242. https://doi.org/10.1590/1678-77572016-0398.

11- Dahl JE, Pallesen U. Tooth bleaching - a critical review of the biological aspects. Crit Rev Oral Biol Med. 2003;14(4):292-304. https://doi.org/10.1177/154411130301400406.

12- Pedrollo Lise D, Siedschlag G, Bernardon JK, Baratieri LN. Randomized clinical trial of 2 nonvital tooth bleaching techniques: A 1-year follow-up. J Prosthet Dent. 2018 Jan;119(1):53-59. https://doi.org/10.1016/j.prosdent.2017.03.004.

13- Paravina RD, Ghinea R, Herrera LJ, Bona AD, Igiel C, Linninger M, Sakai M, Takahashi H, Tashkandi E, Perez Mdel M. Color difference thresholds in dentistry. J

Esthet Restor Dent. 2015 Mar-Apr;27 Suppl 1:S1-9. https://doi.org/10.1111/jerd.12149.

14- Sharma G, Wu W, Dalal EN. The CIEDE2000 color-difference formula: Implementation notes, supplementary test data, and mathematical observations. Color Res Appl. 2005;30(1):21-30. https://doi.org/10.1002/col.20070.

15- Kohli MR, Yamaguchi M, Setzer FC, Karabucak B. Spectrophotometric analysis of coronal tooth discoloration induced by various bioceramic cements and other endodontic materials J Endod. 2015 Nov;41(11):1862-6. https://doi.org/10.1016/j.joen.2015.07.003.

16- Sanchez AR, Rogers RS 3rd, Sheridan PJ. Tetracycline and other tetracycline derivative staining of the teeth and oral cavity. Int J Dermatol. 2004 Oct;43(10):709-15. https://doi.org/10.1111/j.1365-4632.2004.02108.x.

17- Bowles WH, Bokmeyer TJ. Staining of adult teeth by minocycline: binding of minocycline by specific proteins. J Esthet Dent. 1997;9(1):30-4. https://doi.org/10.1111/j.1708-8240.1997.tb00913.x.

18- Cheek CC, Heymann HO. Dental and oral discolorations associated with minocycline and other tetracycline analogs. J Esthet Dent. 1999;11(1):43-8. https://doi.org/10.1111/j.1708-8240.1999.tb00375.x.

19- Good ML, Hussey DL. Minocycline: stain devil? Br J Dermatol. 2003 Aug;149(2):237-9. https://doi.org/10.1046/j.1365-2133.2003.05497.x.

20- Maroto M, Barberia E, Planells P, Garcia Godoy F. Dentin bridge formation after mineral trioxide aggregate (MTA) pulpotomies in primary teeth. Am J Dent. 2005 Jun;18(3):151-4.

21- Watts JD, Holt DM, Beeson TJ, Kirkpatrick TC, Rutledge RE. Effects of ph and mixing agents on the temporal setting of tooth-colored and gray mineral trioxide aggregate. J Endod. 2007 Aug;33(8):970-3. https://doi.org/10.1016/j.joen.2007.01.024.

22- Holland R, de Souza V, Nery MJ, Otoboni Filho JA, Bernabe PF, Dezan Junior E. Reaction of rat connective tissue to implanted dentin tubes filled with mineral trioxide aggregate or calcium hydroxide. J Endod. 1999 Mar;25(3):161-6. https://doi.org/10.1016/S0099-2399(99)80134-4.

23- Steffen R, Van Waes H. Understanding mineral trioxide aggregate/portland-cement: a review of literature and background factors. Eur Arch Paediatr Dent. 2009 Jun;10(2):93-7. https://doi.org/10.1007/BF03321608.

24- Camilleri J. Color stability of white mineral trioxide aggregate in contact with hypochlorite solution. J Endod. 2014 Mar;40(3):436-40. https://doi.org/10.1016/j.joen.2013.09.040.

25- Marin PD, Bartold PM, Heithersay GS. Tooth discoloration by blood: an in vitro histochemical study. Endod Dent Traumatol. 1997 Jun;13(3):132-8. https://doi.org/10.1111/j.1600-9657.1997.tb00026.x

26- Watts A, Addy M. Tooth discolouration and staining: a review of the literature. Br Dent J. 2001 Mar 24;190(6):309-16. https://doi.org/10.1038/sj.bdj.4800959a.

27- Rezende M, Loguercio AD, Kossatz S, Reis A. Predictive factors on the efficacy and risk/intensity of tooth sensitivity of dental bleaching: A multi regression and logistic analysis. J Dent. 2016 Feb;45:1-6. https://doi.org/10.1016/j.jdent.2015.11.003.

28- Kwon SR, Wertz PW. Review of the Mechanism of Tooth Whitening. J Esthet Restor Dent. 2015 Sep-Oct;27(5):240-57. https://doi.org/10.1111/jerd.12152.

29- Eimar H, Siciliano R, Abdallah MN, Nader SA, Amin WM, Martinez PP, Celemin A, Cerruti M, Tamimi F. Hydrogen peroxide whitens teeth by oxidizing the organic structure. J Dent. 2012 Dec;40 Suppl 2:e25-33. https://doi.org/10.1016/j.jdent.2012.08.008.

Table 1. Means (standard deviation) of color parameters assessed at baseline (n=10).

Etiologic agents	Color parameters		
	L*	a*	b*
MTA	74.30 (72.4/76.2)	3.08 (2.0/4.2)	21.96 (20.5/23.4)
TAP	74.86 (72.9/76.8)	2.94 (2.1/3.7)	21.84 (20.1/23.4)
Blood	76.04 (73.6/78.5)	3.31 (2.3/4.3)	22.21 (21.4/23.1)
p-values*	0.381	0.824	0.908

* Calculated by one-way ANOVA. MTA - mineral trioxide aggregate; TAP – triple antibiotic paste. CIE L*a*b* system (L*: white/black; a*: red/green; b*: yellow/blue).

Table 2. Means (standard deviation) of color change caused by exposure to different etiologic agents up to 6 months (n = 10).

Etiologic agent	E_{00} according to staining time			Final color parameters		
	1-month	2-month	6-month	L*	a*	b*
MTA	2.47 (1.9/3.0) ^{Bb}	2.14 (1.4/2.9) ^{Bb}	5.83 (5.1/6.6) ^{Aa}	79.89(77.2/82.6)^{a}	3.26 (2.9/3.7) ^{a}	15.51(14.7/16.3)^{a}
TAP	5.25 (4.2/6.3) ^{Aba}	6.74 (5.0/8.5) ^{Aa}	3.94 (3.3/4.6) ^{Bb}	72.83(70.1/74.7)^{b}	0.92 (0.3/1.5) ^{b}	16.7 (15.0/18.4) ^{a}
Blood	2.58 (1.2/3.9) ^{Bb}	3.54(2.0/5.0)^{ABb}	4.57(3.9/5.3)^{Aab}	77.39(75.1/79.7)^{a}	2.86 (2.5/3.2) ^{a}	14.85(13.7/16.0)^{a}

Distinct letters (uppercase for line, lowercase for row) indicate statistical difference ($p < 0.05$). MTA - mineral trioxide aggregate; TAP – triple antibiotic paste. CIE L*a*b* system (L*: white/black; a*: red/green; b*: yellow/blue).

Table 3. Means (standard deviation) of color change achieved during three weeks of walking bleaching (n = 10).

Etiologic agent	E_{00} according to bleaching time			Final color parameters		
	1-week	2-week	3-week	L*	a*	b*
MTA	3.94 (2.8/5.1) ^{Ca}	4.97 (4.0/6.0) ^{Ba}	6.07 (4.8/7.3) ^{Aa}	86.46(84.9/88.1)^{a}	0.84 (0.4/1.3) ^{a}	11.02(10.1/12.0)^{a}
TAP	4.65 (3.8/5.5) ^{Ca}	6.54 (4.3/8.8) ^{Ba}	8.64(6.7/10.6)^{Aa}	82.04(80.1/83.9)^{b}	0.01 (-0.3/0.3) ^{b}	8.82 (7.6/10.1) ^{b}
Blood	3.70 (1.9/5.5) ^{Ca}	5.19 (4.1/6.3) ^{Ba}	6.25 (4.7/7.8) ^{Aa}	84.89 (83.2/86.6)^{a}	1.09 (0.5/1.6) ^{a}	11.08(10.3/11.9)^{a}

Distinct letters (uppercase for line, lowercase for row) indicate statistical difference ($p < 0.05$). MTA - mineral trioxide aggregate; TAP – triple antibiotic paste. CIE L*a*b* system (L*: white/black; a*: red/green; b*: yellow/blue).

Figures

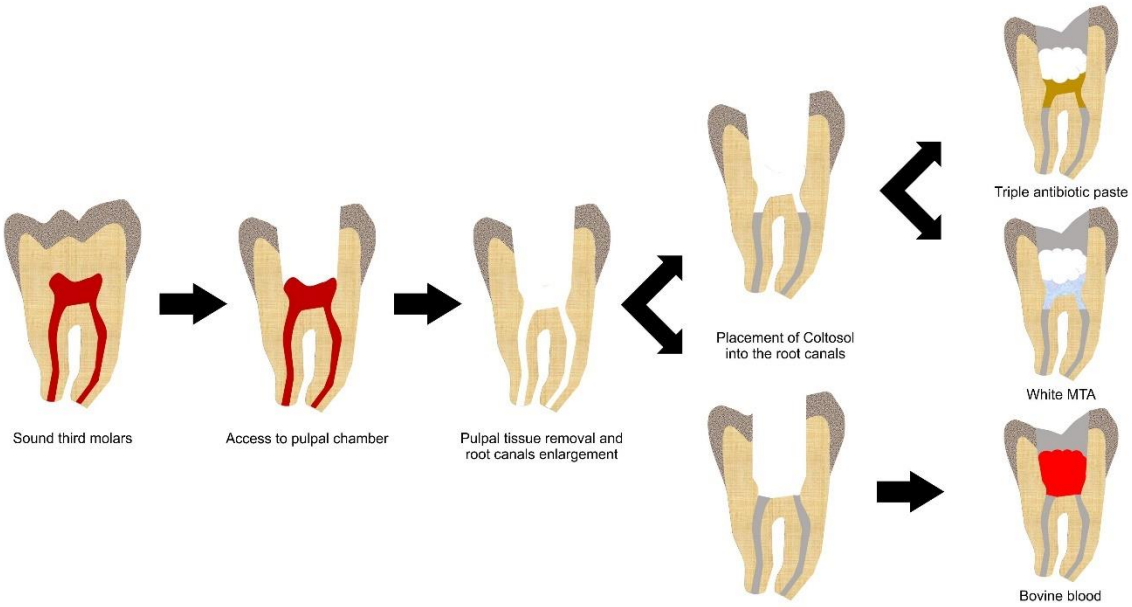

Figure 1. Experimental design presenting the procedures carried out to prepare the specimens and placement of staining agents. MTA - mineral trioxide aggregate.

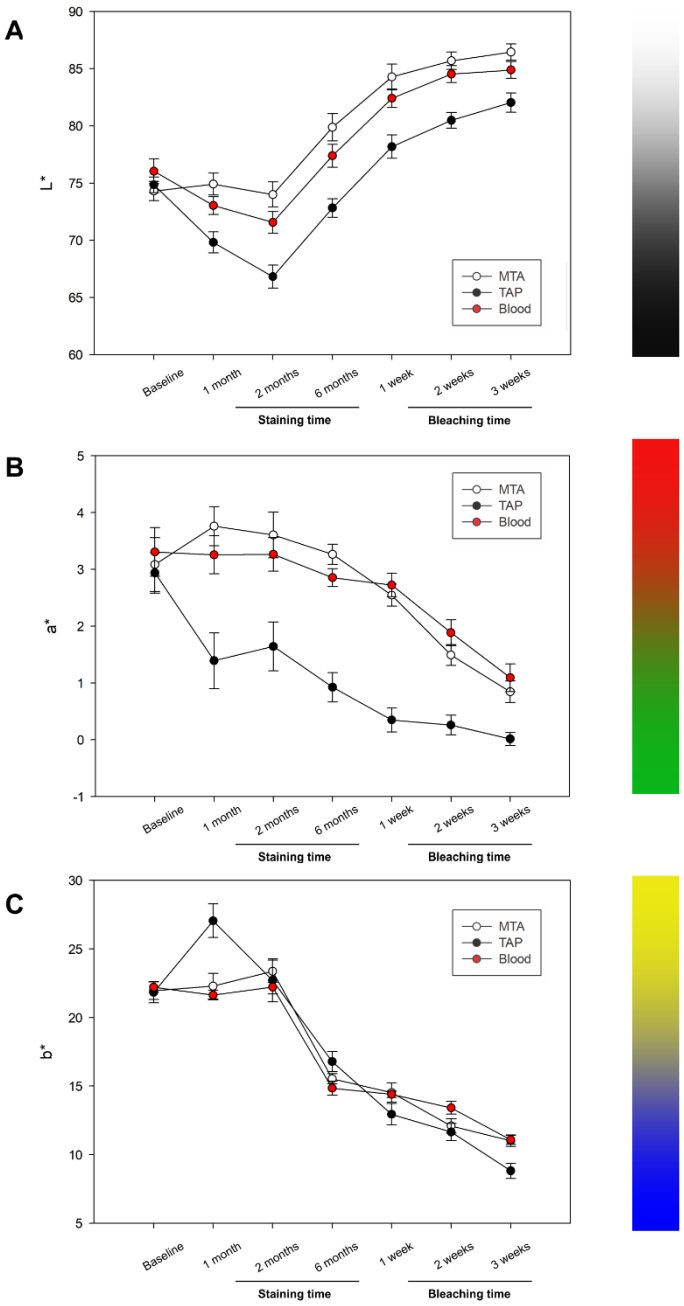

Figure 2. Behavior of color parameters (L* a* b*) during the experiment. (A) parameter L*: white/black; (B) parameter a*: red/green; and (C) parameter b*: yellow/blue. MTA - mineral trioxide aggregate; TAP – triple antibiotic paste.

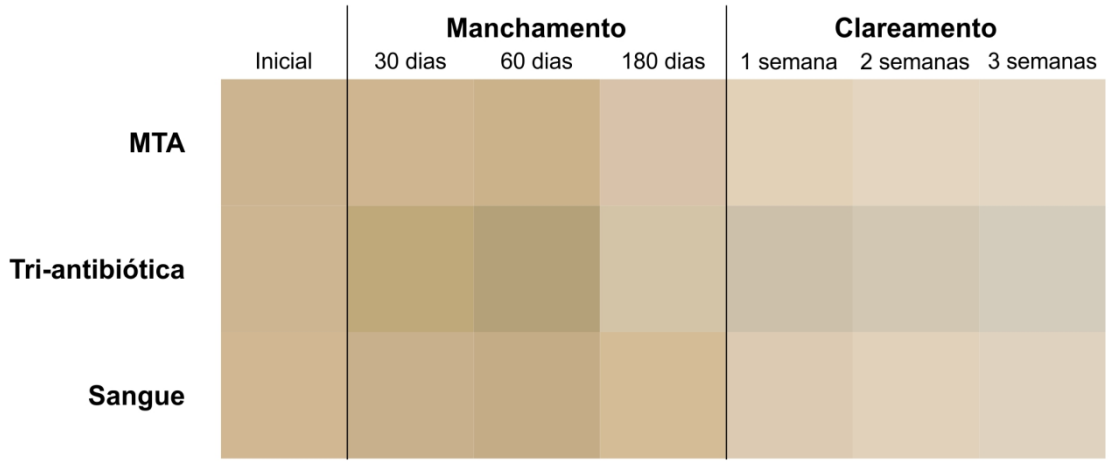

Figure 3. Illustrative square-shaped specimens drawn based on data from L*a*b converted to RGB system demonstrating the color changes of specimens during the experiment. MTA - mineral trioxide aggregate; TAP – triple antibiotic paste.

5-CONSIDERAÇÕES FINAIS

Em conclusão, dentre os materiais endodônticos utilizados na terapia regenerativa, a pasta tri-antibiótica resultou em descoloração dentária mais significativa. O clareamento interno resultou na melhora da cor dos dentes sem diferença na mudança de cor entre os agentes etiológicos, mas os dentes expostos à pasta tri-antibiótica permaneceram com descoloração mais acentuada mesmo após os procedimentos de clareamento.

6-COMUNICADO A IMPRENSA

Em casos de infecção dentária em dentes não completamente formados com comprometimento da polpa, a terapia regenerativa pode ser aplicada. Esta consiste no uso de uma medicação antibiótica dentro do canal afim de reduzir a infecção e permitir a continuação da formação da raiz. Entretanto, as medicações mais comumente utilizadas para este fim podem induzir o escurecimento do dente. Tendo em vista, que a estética é um fator primordial na odontologia, e o escurecimento de um dente pode ter um impacto significativo na qualidade de vida do paciente. Baseado nisso, uma dissertação de mestrado realizada no Programa de Pós-graduação em Odontologia da Universidade Federal de Sergipe buscou avaliar o nível de escurecimento provocado por duas medicações usadas para este fim; e efetividade do clareamento dental realizado em seguida. As medicações foram colocadas em molares extraídos e a mudança de cor foi avaliada durante 6 meses. Em seguida, os dentes foram clareados por 21 dias e a alteração de cor também avaliada semanalmente. Os resultados revelaram que a pasta tri-antibiótica (que associa três diferentes antibióticos) apresentou descoloração dentária mais significativa e que a pior cor que permaneceu após os procedimentos de clareamento dentário. Entretanto, ressalta-se, que o clareamento dental resultou na melhora da cor dos dentes, independentemente da causa do escurecimento.

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REFERÊNCIAS

1- Lenherr P, Allgayer N, Weiger R, Filippi A, Attin T, Krastl G. Tooth discoloration induced by endodontic materials: a laboratory study. Int Endod J 2012; 45:942-9.

2- Plotino G, Buono L, Grande NM, Pameijer CH, Somma F. Nonvital tooth bleaching: a review of the literature and clinical procedures. J Endod 2008; 34:394-407.

3- Arens D. The role of bleaching in esthetics. Dent Clin North Am 1989; 33:319–336.

4- Marin PD, Bartold PM, Heithersay GS. Tooth discoloration by blood: an in vitro histochemical study. Endod Dent Traumatol 1997; 13:132-8.

5- Krastl G, Allgayer N, Lenherr P, Filippi A, Taneja P, Weiger R. Tooth discoloration induced by endodontic materials: a literature review. Dental Traumatology 2013; 29:2–7.

6- Wingler R, Kaufman AY, Lin S, Steinbock N, Hazan-Molina H, Torneck CD. Revascularization: A Treatment for Permanent Teeth with Necrotic Pulp and Incomplete Root Development. J Endod 2013; 39:319-26.

7- Cao Y, Song M, Kim E, Shon W, Chugal N, Bogen G, Lin L, Kim RH, Park NH, Kang MK. Pulp-dentin Regeneration: Current State and Future Prospects. J Dent Res 2015; 94:1544-51.

8- Kim JH, Kim Y, Shin SJ, Park JW, Jung IY. Tooth Discoloration of Immature Permanent Incisor Associated with Triple Antibiotic Therapy: A Case Report. J Endod 2010; 36:1086-91.

9- Marconyak LJ Jr, Kirkpatrick TC, Roberts HW, Roberts MD, Aparicio A, Himel VT, Sabey KA. A Comparison of Coronal Tooth Discoloration Elicited by Various Endodontic Reparative Materials. J Endod 2016; 42: 470-3.

10- Santos LG, Felippe WT, Souza BD, Konrath AC, Cordeiro MM, Felippe MC. Crown discoloration promoted by materials used in regenerative endodontic procedures and effect of dental bleaching: spectrophotometric analysis. J Appl Oral Sci 2017; 25:234-42.

11- Dahl JE, Pallesen U. Tooth bleaching--a critical review of the biological aspects. Crit Rev Oral Biol Med 2003; 14:292-304.

12- Algahtani MQ. Tooth-bleaching procedures and their controversial effects: A literature review. Saudi Dent J 2014; 26:33–46.

13- Paravina RD, Ghinea R, Herrera LJ, Bona AD, Igiel C, Linninger M, Sakai M, Takahashi H, Tashkandi E, Perez Mdel M. Color difference thresholds in dentistry. J Esthet Restor Dent 2015; 27:S1-9.

14- Sharma G, Wu W, Dalal EN. The CIEDE2000 color-difference formula: Implementation notes, supplementary test data, and mathematical observations. Color Res Appl 2005; 30:21-30.