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Tooth discoloration caused by repair cements in a simulated regenerative endodontic procedure

Thayná Regina Pelissari[1*], Mônica Pagliarini Buligon[1], Carlos Eduardo Victor da Costa Ribeiro[1], Renata Dornelles Morgental[1,2], Liliana Gressler May[3], Carlos Alexandre Souza Bier[1,2], Claudia Medianeira Londero Pagliarin[1,2]

[1] Department of Stomatology, Postgraduate Program in Dentistry, Emphasis on Endodontics, Federal University of Santa Maria (UFSM), Santa Maria, Rio Grande do Sul, Brazil.

[2] Department of Stomatology, School of Dentistry, Federal University of Santa Maria (UFSM), Santa Maria, Rio Grande do Sul, Brazil.

[3] Department of Restorative Dentistry, School of Dentistry, Federal University of Santa Maria (UFSM), Santa Maria, Rio Grande do Sul, Brazil.

Corresponding author:
Thayná Regina Pelissari
Roraima Avenue, 1000, T Street, Building 26F, Room 2383.
Zip Code: 97015900. Santa Maria, Rio Grande do Sul, Brazil.
Telephone: +55 (055) 3220-9210.
E-mail: thaynapelissari@gmail.com

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Aim: The present study evaluated color changes caused by endodontic repair materials in a simulated regenerative endodontic procedure (REP). **Methods:** Fifty-six bovine incisors were randomly distributed into three experimental groups according to the repair cement placed over the blood clot [Conventional white MTA (Angelus), MTA Repair HP (Angelus), and Bio-C Repair (Angelus)] and a control group (n=14). Color measurements were taken before the access cavity preparation, one week, one month, three months, and six months after REP. The CIELAB system was chosen to calculate color change (ΔE), and the generalized estimating equations model was used to compare mean ΔE values between cements and periods. **Results:** Teeth from the three cement groups exhibited a clinically perceptible color change. No significant differences between groups were found in any experimental period. Within each group, MTA Repair HP and Bio-C Repair exhibited significantly greater color change at the six-month evaluation. **Conclusions:** The three endodontic repair materials caused similar changes in tooth color when used as a cervical plug in a simulated REP.

Keywords: Calcium compounds. Silicates. Regenerative endodontics. Tooth discoloration.

Introduction

Regenerative endodontic procedures (REP) seem to be a viable clinical approach for the treatment of immature teeth with pulp necrosis and apical periodontitis, as demonstrated by studies using animal models[1,2] and several cases reports available in the literature[3-5]. These therapies enable the continuation of root development in both length and thickness, reinforce the root structure, and reduce the risk of fracture when compared to the traditional apexification procedure[3,4]. Other advantages include the shorter time required to complete the treatment and the lower cost for the patient[6].

The key factor for a favorable outcome using this new treatment approach is the effective control of infection within the root canal[2]. Chemomechanical root canal preparation can significantly reduce the number of microorganisms but does not eliminate them[2]. Thus, a proper antimicrobial protocol must be used to ensure adequate periapical healing[5].

According to the guidelines of the American Association of Endodontists, after the formation of a blood clot in the root canal, cervical sealing must be performed with an adequate material, confined to 2 to 3 mm below the cementoenamel junction (CEJ)[7]. Bioceramic materials are widely used to seal blood clots after REP[8]. In addition to biocompatibility and the induction of the formation of hard tissue, these materials also have considerable sealing capacity[9].

Mineral trioxide aggregate (MTA) was introduced on the market in 1999 in its grey version (ProRoot MTA; Dentsply Tulsa Dental Specialties, Johnson City, TN). The white form was available only in 2002[10]. White MTA was intended to solve the compromised aesthetics related to the use of this material[11]. However, several *in vitro* and clinical studies continued to demonstrate tooth discoloration when this cement maintains prolonged contact with the dentin[12-16]. The radiopacifier bismuth oxide had not been altered, and it was later implicated as responsible for the tooth discoloration in both MTA formulations[13].

New bioceramic formulations such as MTA Repair HP and Bio-C Repair (Angelus, Londrina, PR, Brazil) are currently available and have different radiopacifiers in their formulation (calcium tungstate and zirconia oxide, respectively). According to the manufacturer, these products do not promote tooth discoloration. However, this claim has not yet been confirmed in studies. Therefore, the present study aimed to determine whether the endodontic repair materials – conventional white MTA-Angelus, MTA Repair HP, and Bio-C Repair – promote a color change when used as cervical sealing in a simulated REP. The null hypothesis is that the different cements do not promote clinically noticeable tooth discoloration ($\Delta E > 2.7$)[15].

Methods

Ethical considerations

The present study employed human blood donated by the hemotherapy sector of the institution. The study received approval from the institutional review board (certificate number: 24525319.2.0000.5346). The bovine teeth were donated by a slaughterhouse and were taken from animals killed for commercial reasons.

Sample size and tooth preparation

Fifty-six extracted lower bovine incisors were used in this study. The calculation of the sample size was performed in the software OpenEpi 3.01, considering the parameters described by Jang et al.[17]: mean ΔE in group 1: 14.85 with a standard deviation of 6.36; mean ΔE in group 2: 9.11 with a standard deviation of 4.07. Accordingly, for the analysis with an alpha=0.05 and 80% testing power, a total of 56 specimens (fourteen teeth per test group) were indicated as the minimum size required for observing significant differences.

The teeth were previously inspected. Those with root resorption, root canal obliteration, more than one root canal, incompatible size, or intrinsic discoloration were excluded. The selected teeth were cleaned with an ultrasonic dental scaler (Schuster, Santa Maria, RS, Brazil) and stored in distilled water under refrigeration until use. The apical portion of each specimen was sectioned 15-mm below the CEJ, and the apical opening was sealed with a temporary restorative material (CaiTHEC, São José dos Pinhais, PR, Brazil) in the apical 4 mm of the root canal. After preparing the access cavity, passive irrigation was performed with 1.5% sodium hypochlorite solution (NaOCl) (Dermapelle, Santa Maria, RS, Brazil). The root canals were then prepared with Gates-Glidden drills #3 to #6 (Dentsply Sirona, York, PA, USA) to standardize the diameter, as described by Shokouhinejad et al.[18].

Calcium hydroxide powder (Biodental, Criciúma, SC, Brazil) associated with saline solution was used as intracanal medication, with the root canals filled below the CEJ. Sealing was performed with a cotton pellet and temporary restorative material (CaiTHEC, São José dos Pinhais, PR, Brazil) in the pulp chamber. Next, the teeth were incubated at 37ºC in a humidity-controlled container for two weeks.

Experimental setup

After two weeks, the temporary pulp chamber seal was removed, and the root canals were irrigated with 1.5% NaOCl solution (Dermapelle, Santa Maria, RS, Brazil), followed by 20 ml of 17% EDTA (Biodinâmica, Ibiporã, PR, Brazil) for 5 minutes. Final irrigation was performed with saline solution. The canals were dried with absorbent paper points (Tanari, Manaus, AM, Brazil). Then, the teeth (n = 14/group) were randomly distributed into three experimental groups (Conventional white MTA-Angelus, MTA Repair HP, and Bio-C Repair) and a control group (Table 1). The root canals in the control group were not filled with any material. In the experimental groups, small pieces of hemostatic sponge (Maquira, Maringá, PR, Brazil) were placed in the root canals, and human blood was then introduced with a syringe to simulate the induction of bleeding and formation of a blood clot, filling the root canal to 4 mm below the CEJ[19]. After, 3-mm plugs of the respective materials were gently placed into the specimens below the CEJ to seal the blood clot.

Each material was prepared according to the manufacturer's instructions (Angelus, Londrina, PR, Brazil). Conventional white MTA was mixed with distilled water for 30s on a sterilized glass tray (proportion: 1 sachet/1drop distilled water), MTA Repair HP was mixed with distilled water for 40 s on a sterilized glass tray (proportion: 1sachet/2 drops distilled water) and Bio-C Repair was ready-for-use, not requiring manipulation.

The filling level was measured with a periodontal probe (Golgran, São Caetano do Sul, SP, Brazil) to define the correct position and preserve the space below the CEJ. The coronal access was cleaned with cotton pellets soaked in ethyl alcohol and sealed with temporary restorative material (CaiTHEC, São José dos Pinhais, PR, Brazil).

Table 1. Materials used in experimental groups and respective compositions.

Group	N	Material	Manufacturer	Composition
1	14	Conventional MTA	Angelus, Brazil	Tricalcium silicate Dicalcium silicate Tricalcium aluminate Calcium oxide Calcium tungstate Distilled water + plasticizer
2	14	MTA Repair HP	Angelus, Brazil	Tricalcium silicate Dicalcium silicate Tricalcium aluminate Calcium oxide Calcium tungstate Distilled water + plasticizer
3	14	Bio-C Repair	Angelus, Brazil	Tricalcium silicate Dicalcium silicate Tricalcium aluminate Calcium oxide Silicon oxide Iron oxide Zirconia oxide Dispersing agents
4	14	Control	-	No filling

After this experimental setup, the teeth remained stored in individual flasks immersed in distilled water at 37ºC to simulate human body temperature throughout the experimental period (1 week, 1, 3 and 6 months).

Tooth color assessment

The change in tooth color was measured with a portable digital spectrophotometer (VITA Easyshade compact; VITA Zahnfabrik, Bad Säckingen, Germany) following the recommendations of the International Commission on Illumination. The spectrophotometer was previously calibrated – before each measurement – according to the manufacturer’s instructions. A single operator who had undergone training and calibration exercises performed all color evaluations in the same environment (darkroom). The measurement site on the crown was standardized for each tooth using custom-made acrylic matrices with a 6-mm diameter opening in the cervical third of the buccal face of the crown, 1-mm above the tooth neck. Color measurements were taken in triplicate, and the mean was calculated.

All specimens were submitted to an initial color evaluation before coronal access (T0 – baseline). Other assessments were performed one week (T1), one month (T2), three months (T3), and six months (T4) after REP. The teeth were stored in individual flasks in an incubator at 37ºC between evaluation times. Color measurement was performed according to the CIELAB system, which expresses color in three coordinates: L* refers to lightness, ranging from 0 (black) to 100 (white), while a* and b* are chroma coordinates: a* from green (-) to red (+) and b* from blue (-) to yellow (+). The change in tooth color over the four evaluation times was expressed by ΔE. A color change is considered perceptible to the human eye when ΔE is equal to or greater than 2.7[15]. The total color difference (ΔE) of the specimens was calculated using the following equation:

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

Statistical analysis

The data from the different groups were expressed as mean and standard deviation values. The Shapiro-Wilk test was used to determine data distribution (normal or non-normal). Generalized estimating equations (GEE) were used to compare mean ΔE values between cements and periods. The GEE model was composed of an unstructured work correlation matrix, a robust estimator covariance matrix, and normal distribution with an identity link function. When significant differences were detected, the Bonferroni post-hoc test was used to identify the different categories. All analyses were performed using the IBM SPSS Statistics v.25 software (IBM SPSS Inc., Chicago, IL, USA). The significance level was set at 5%.

Results

Mean ΔE values for the different experimental groups and control are shown in Table 2. The teeth treated with the three endodontic repair materials underwent clinically perceptible color changes (ΔE> 2.7).

Table 2. CIELAB analyses: Comparison of main effects and interaction (cement*time) on ΔE.

	Cement				TOTAL
	MTA	MTA-HP	BIO-C	CONTROL	
	Mean [95% CI]	**Mean [95% CI]**	**Mean [95% CI]**	**Mean [95% CI]**	**Mean [95% CI]**
ΔE 1 (1 week)	6.1Aa [3.6; 8.6]	4.0Aa [3.1; 5.0]	5.2Aa [3.6; 6.9]	6.4Aa [4.8; 8.00]	5.4 [4.6; 6.3]
ΔE 2 (1 month)	4.8Aa [3.3; 6.4]	3.9Aa [3.1; 4.6]	5.1Aa [3.5; 6.7]	3.4Ab [2.2; 4.6]	4.3 [3.6; 5.0]
ΔE 3 (3 months)	5.5Aa [3.7; 7.4]	3.7Aa [3.0; 4.5]	5.0Aa [3.3; 6.8]	6.5Aa [4.8; 8.1]	5.2 [4.4; 6.0]
ΔE 4 (6 months)	7.0Aa [5.2; 8.8]	9.5Ab [7.6; 11.4]	8.2Ab [6.1; 10.3]	6.0Aa [4.6; 7.4]	7.7 [6.8; 8.6]
TOTAL	5.9 [3.9; 7.8]	5.3 [4.2; 6.4]	5.9 [4.1; 7.7]	5.5 [4.1; 7.0]	

*GEE Model.
Cement (P=0.957); Time (P<0.001); Interaction (P<0.001).
Color differences (ΔE) were compared to baseline and calculated based on parameters L*, a* e b*, using CIELab scale.
The following comparisons were made: ΔE1 = 1 week vs. before coronal access (baseline); ΔE2 = 1 month vs. baseline; ΔE3 = 3 months vs. baseline; ΔE4 = 6 months vs. baseline.
Similar uppercase letters indicate similar mean ΔE among cement groups (rows).
Similar lowercase letters indicate similar mean ΔE among evaluation times (columns).

The interaction between cement and time had a significant effect on ΔE ($P < .05$). When fixing the color change of different cements and comparing means among the different times, significant differences were found at the six-month evaluation (ΔE4) in the MTA Repair HP and Bio-C Repair groups; the behavior of MTA Repair HP and Bio-C Repair cements changed six months after REP. Also, a significant difference was found in the control group at the one-month evaluation (ΔE2); mean ΔE2 values were lower than the means found at the other evaluations.

No statistically significant differences among cements were found at any evaluation time ($P > .05$).

Discussion

White MTA has been considered the “gold-standard” material for REP, due to its biocompatibility properties, sealing capacity, and marginal adaptation. However, this substance has the potential for discoloration of the dental structure[20]. Tooth discoloration is an undesirable outcome associated with the use of endodontic repair materials for cervical sealing during REP[18] and can significantly impact the patient’s quality of life[21]. Thus, it is necessary to investigate new bioactive repair materials with similar sealing and biological properties to those of MTA but without such a disadvantage[9,12]. The present study aimed to evaluate the color change induced by three bioactive repair cements used for cervical sealing after simulated REP. The null hypothesis was accepted since no significant differences between cements were found at any evaluation time.

The observed similarity in the discoloration potential between cements can be partially explained by the similarity of the composition of the products. The new endodontic repair materials have formulations free of bismuth oxide, which may be responsible for severe discoloration of dental surfaces due to its interaction with collagen in the dentin matrix[13]. The conventional MTA replaced bismuth oxide for calcium tungstate; the MTA Repair HP was already formulated with calcium tungstate as radiopacifier; while Bio-C Repair contains zirconia oxide. Moreover, Bio-C Repair is sold as “ready-to-use” – this facilitates handling and reducing clinical time. This new product has demonstrated excellent cytocompatibility and biomineralization potential in *in vitro* studies[22,23], as previously established for MTA.

The three endodontic repair materials analyzed promoted clinically noticeable color change – that is, factors beyond the radiopacifier may contribute to tooth discoloration. This contrasts the findings reported by Marciano et al.[13], who attributed the color change to the interaction between bismuth oxide and collagen in the dentin matrix. Additionally, the cements showed similar behavior at the different evaluation times. However, when fixing the “cement” variable and analyzing the “time”, we found a statistically significant difference in the Bio-C Repair and MTA Repair HP groups at the six-month evaluation. This may be explained because both cements contain heavy metal ions, such as aluminum or iron. A recent study suggested that the metal constituents of the cement may have staining potential[24]. The oxidation of the iron remaining in the set material serves as a potential mechanism for tooth discoloration over the long term[14,24,25].

A similar color change was found in the control group when compared to the three experimental ones, differently from what has been observed by other authors[12,13]. Madani et al.[19] showed that the effect of coronal access could change tooth color, as a slight change was observed after coronal preparation. This difference in translucency could explain the color change found in the control group in the present study.

The change in tooth color found after using MTA may be exacerbated due to the interaction between the cement and red blood cells in the clot resulting from the induction of bleeding into the root canal[14,26]. Due to the physiological degradation of blood erythrocytes in contact with the cement, the reddish hue becomes dark brown over time, resulting in the darkening of the cement and tooth discoloration in the long term[14,26]. Furthermore, the hydration process of MTA is slow and could favor the absorption of erythrocytes, causing greater color changes[14,25]. Several studies in the literature have found that tooth discoloration associated with the use of calcium-silicate cements increases considerably with blood contamination, whether in the presence or absence of bismuth oxide[14,18,19,25-27].

An increase in tooth discoloration (ΔE) occurred in all groups over time. A similar finding was reported in a study comparing the effect of endodontic repair cements on tooth color change in the presence and absence of blood[19]. Furthermore, the material's porosity is another aspect to consider, as the absorption of blood components in the pores and gaps of the cements can contribute to discoloration[25-27].

There are also reports that the irrigating solution can influence the color change of calcium silicate-based materials[20,28]. Even products without bismuth oxide exhibited clinically perceptible discoloration when immersed in sodium hypochlorite and chlorhexidine gluconate, whereas distilled water did not cause noticeable discoloration of any material[28].

The use of calcium hydroxide associated with saline as an intracanal medication is effective at disinfecting the root canal and preventing bacterial growth[29]. It also avoids significant dental discoloration, unlike what happens with the triple antibiotic paste[27], which could lead to misinterpretation of the study data. According to the protocol of the American Association of Endodontists, calcium hydroxide is indicated as an alternative intracanal medication in REP and is a good substitute for antibiotic compounds[7]. Thus, calcium hydroxide was chosen in the present study instead of the triple antibiotic paste, thereby avoiding major aesthetic problems, many of which are irreversible.

Bovine teeth were used instead of human teeth in order to improve the standardization of the sample and to have a large flat surface for color analysis, beyond considering ethical issues and difficulties in obtaining a sufficient quantity of human specimens free of cavities and/or restorations nowadays[18,24,26]. Bovine coronal dentin has similar tubular density and diameter to human coronal dentin[30]. Therefore, laboratory experiments involving the tooth crown can be properly performed with bovine teeth[29].

Although well designed, *in vitro* studies have inherent limitations. Thus, the results of laboratory studies should be carefully extrapolated to clinical practice since different mechanisms may interfere in the clinical scenario. In this *in vitro* study, which simulated the clinical situation of REP, all the new cements tested had the potential to promote clinically perceptible tooth discoloration when used as the cervical plug, and the tooth color change was more significant in the long term (after six months). These results reveal that the mechanisms by which repair endodontic cements impact coronal tooth discoloration remain unknown and beyond the type of radiopacifier present in the composition. Further investigations are required.

In conclusion, the three endodontic repair materials caused similar changes in tooth color when used as a cervical plug in a simulated REP. For MTA Repair HP and Bio-C Repair cements, color changes intensified after 6 months.

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Data availability

Datasets related to this article will be available upon request to the corresponding author.

Conflict of interest

The authors declare no conflict of interests.

Author Contribution

Thayná Regina Pelissari: Conceptualization; Data curation; Investigation; Methodology; Formal analysis; Visualization; Writing – original draft; Writing – review and editing. **Mônica Pagliarini Buligon**: Data curation; Investigation; Methodology; Writing – review and editing. **Carlos Eduardo Victor da Costa Ribeiro:** Data curation; Methodology; Formal analysis; Writing – review and editing. **Renata Dornelles Morgental**: Conceptualization; Methodology; Supervision; Writing – review and editing. **Liliana Gressler May**: Data curation; Methodology; Visualization; Writing – review and editing. **Carlos Alexandre Souza Bier**: Metodhology; Supervision; Writing – review and editing. **Claudia Medianeira Londero Pagliarin:** Conceptualization; Investigation; Methodology; Supervision; Writing – review and editing. All authors actively participated in discussing the manuscript's findings and have revised and approved the final version of the manuscript.

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