

Ex vivo tooth discoloration induced by calcium silicate-based materials: analysis of an experimental cement

Descoloração dentária *ex vivo* induzida por materiais à base de silicato de cálcio: análise de um cimento experimental

Suyane Maria LUNA-CRUZ^{1,2} , Bernardo Almeida AGUIAR¹ , Ana Grasiela LIMOEIRO³ , Marco Antônio Húngaro DUARTE³ , Bruno Carvalho de VASCONCELOS¹ , Juliano Sartori MENDONÇA¹ 

1 - Universidade Federal do Ceará, Programa de Pós-graduação em Odontologia. Fortaleza, CE, Brazil.

2 - Centro Universitário UniFametro. Fortaleza, CE, Brazil.

3 - Universidade de São Paulo, Faculdade de Odontologia de Bauru, Departamento de Dentística, Endodontia e Materiais Odontológicos. Bauru, SP, Brazil.

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ABSTRACT

Objective: This study aimed to investigate of bioactive materials with specific properties, particularly highly plastic bioceramic cements. These materials are being studied extensively due to their potential to maintain pulp vitality and promote tissue regeneration. **Material and Methods:** Tooth discoloration induced by an experimental tricalcium silicate-based cement (EC) was evaluated and compared with that of Biodentine (BD) and white MTA-Angelus (MTA). Cavities were prepared on the lingual surfaces of thirty-two blocks of healthy bovine incisors. The blocks were chemically cleaned and then subjected to an initial color evaluation (CIELab values) using a spectrophotometer and randomly divided into three experimental groups (n=10); two additional blocks served as controls. After manipulation, the cements were placed in the cavities, which were subsequently restored with composite restorations. After another color measurement (baseline), they were immersed in bottles of distilled water; they were stored at 37 °C and 100% humidity for the entire test period. The color change (ΔE) was measured after 14, 30, 120 and 150 days. ANOVA and Tukey tests showed significant differences after 14 days (EC vs. MTA), 30 days (EC vs. BD) and 120/150 days (EC vs. BD/MTA) ($p < 0.05$). **Results:** All tested materials induced ΔE changes, with the EC group showing the least change at the end of the experiment ($\Delta E=4.08$). **Conclusion:** EC induced less color change over a 5-month period and thus showed color stability over the entire period, whereas BD and MTA showed progressive discoloration.

KEYWORDS

Dental cements; Dental materials; Endodontics; Silicate cement; Tooth discoloration.

RESUMO

Objetivo: O objetivo deste estudo foi investigar materiais bioativos com propriedades específicas, particularmente cimentos biocerâmicos altamente plásticos. Esses materiais estão sendo amplamente estudados devido ao seu potencial para manter a vitalidade da polpa e promover a regeneração dos tecidos. **Material e Métodos:** A descoloração dentária induzida por um cimento experimental à base de silicato tricálcico (CE) foi avaliada e comparada com a do Biodentine (BD) e do MTA-Angelus branco (MTA). Foram preparadas cavidades nas superfícies linguais de trinta e dois blocos de incisivos bovinos saudáveis. Os blocos foram quimicamente limpos e, em seguida, submetidos a uma avaliação inicial de cor (valores CIELab) usando um espectrofotômetro e divididos aleatoriamente em três grupos experimentais (n=10); dois blocos adicionais serviram como controles. Após a manipulação, os cimentos foram colocados nas cavidades, que foram posteriormente restauradas com compósito. Após outra medição de cor (valor de referência), eles foram imersos em frascos de água destilada;

foram armazenados a 37 °C e 100% de umidade durante todo o período de teste. A alteração de cor (ΔE) foi medida após 14, 30, 120 e 150 dias. Os testes ANOVA e Tukey mostraram diferenças significativas após 14 dias (CE vs. MTA), 30 dias (CE vs. BD) e 120/150 dias (CE vs. BD/MTA) ($p < 0,05$). **Resultados:** Todos os materiais testados induziram alterações de ΔE , sendo que o grupo EC apresentou a menor alteração no final do experimento ($\Delta E=4,08$). **Conclusão:** O EC induziu menos alterações de cor em um período de 5 meses e, portanto, apresentou estabilidade de cor durante todo o período, enquanto o BD e o MTA apresentaram descoloração progressiva.

PALAVRAS-CHAVE

Cimentos odontológicos; Materiais odontológicos; Endodontia; Cimento de silicato; Descoloração dos dentes.

INTRODUCTION

The quest for conservative dental treatments that preserve pulp vitality and regenerate biological tissues has prompted research into bioactive materials with these properties and the development of highly plastic bioceramic cement [1-3]. Most studies in the literature are based on the evaluation of commercially available calcium silicate cements, which are indicated as direct and indirect pulp capping and endodontic repair materials [4-8]. However, some of these materials have limitations regarding their clinical indication, such as the increased risk of tooth discoloration [4,9-12], which becomes more relevant as these agents can be used at levels up to the cervical region [9,13].

Biodentine (BD; Septodont, Saint-Maur-des-Fossés, France) consists of a powder of tricalcium silicate, zirconium oxide, calcium oxide, calcium carbonate, red and yellow pigments, and brown iron oxide; the liquid is composed of water, calcium chloride and a thickening agent. In turn, the white MTA-Angelus (MTA; Angelus Ind. Prod. Odont. S/A, Londrina, PR, Brazil) presents tricalcium and dicalcium silicates, tricalcium aluminate (white Portland cement), calcium oxide and calcium tungstate in its powder and

only distilled water in its liquid. The powder of the experimental calcium silicate-based cement (EC), on the other hand, consists of tricalcium silicate, zirconium oxide, calcium oxide and calcium phosphate. Its liquid contains water, a water-soluble polymer, and a setting accelerator (Table I).

Originally, bismuth oxide was added to these cements because it was known as a radiopacifier [14]. However, both clinical and experimental observations showed an increase in tooth discoloration associated with the presence of this compound BAHA [15,16]. This process occurs due to the interaction between bismuth oxide and collagen in the absence of oxygen, resulting in a dark-colored precipitate that causes pigmentation of the dentin [17-19].

The increasing demand for aesthetics [20], where conspicuous changes in tooth color could be considered a treatment failure, has led to the search for alternative radiopacifiers. Compounds such as zirconium oxide and calcium tungstate are already available in some commercial products [4,19,21], and, more recently, niobium oxide [22] and ytterbium oxide [23] have been evaluated for the same purpose.

Table I - Chemical composition of the cements used

	BD (#B28191)	MTA (#101663)	EC (Requested Patent)
Powder	Tricalcium silicate	Tricalcium silicate	Tricalcium silicate
	Zirconium oxide	Dicalcium silicate	Zirconium oxide
	Calcium oxide	Tricalcium aluminate	Calcium oxide
	Calcium carbonate	Calcium oxide	Calcium phosphate
	Yellow pigment	Calcium tungstate	
	Red pigment		
	Brown iron oxide		
Liquid	Water	Distilled water	Ultrapure water
	Calcium chlorite dehydrate		Water soluble polymers
	Areo		Setting accelerator

However, in addition to satisfactory radiopacity properties, these new materials must also have compatible physicochemical and biological properties while preserving the color of the tooth structures. Considering that any new material indicated as a capping or repair agent should not produce a noticeable color change, the present study compared tooth discoloration by an experimental calcium silicate-based cement with that of two commercial cements, Biodentine and white MTA-Angelus. The null hypothesis was that there would be no significant color change between the materials.

MATERIAL AND METHODS

For the study, the sample size calculation was based on the results of a previous study [24] (means of 1.96 to 4.91), considering an alpha error of 5%, a power of 80%, and an allocation ratio of $N2/N1 = 1$, resulting in a sample size of 10 specimens per group ($n = 10$).

Thirty-two sound bovine incisors were therefore collected and enamel/dentin blocks measuring 10 x 10 x 3.5 mm (height x width x thickness) were prepared. Circular cavities with a diameter of 5.0 mm and a depth of 1.5 mm were then prepared on the lingual surfaces using high-speed diamond burs (#4054, KG Sorensen, Cotia, Brazil) with copious irrigation. Blocks with residual enamel/dentin less than 2.0 mm were replaced.

After completion of the cavities, the blocks were placed in an ultrasonic bath where they were successively bathed with a 2.5% sodium hypochlorite solution (Biodinamica, Ibiporã, PR, Brazil) and 17% ethylenediaminetetraacetic acid (Biodinamica) [17]. The samples were then washed with saline and carefully dried with sterile gauze. At this stage, the initial color of the blocks was measured using a clinical spectrophotometer (Vita EasyShade V, VITA Zahnfabrik, Bad Sackingen, Germany) under controlled light to verify the initial values and ensure homogeneity of the samples in the groups. The measurements were based on the CIELab (International Commission on Illumination) color space values, represented by $L^*a^*b^*$, where “L” stands for luminosity, “a” for measurements on the red-green axis and “b” for measurements on the yellow-blue axis [17]. Samples whose “L” values deviated by more than 10% from the overall mean value were also replaced.

The initial color parameters of the samples allowed random grouping according to the cements used. All tested materials contain calcium silicates as active ingredients, with some variations nowadays.

The margins of the cavities were etched with 37% phosphoric acid (Condac 37; FGM Dental Group, Joinville, SC, Brazil) for 30 seconds, followed by extensive washing for 30 seconds [15,17]. Two layers of dentin adhesive (Single Bond 2, 3M ESPE, Sumaré, Brazil) were then applied to the same cavity margins. After applying the second layer (Optilight LD Max, Gnatus, Ribeirão Preto, Brazil), a gentle blow-out and photopolymerisation for 20 s were performed.

The commercial cements BD and MTA were mixed according to the manufacturer's instructions. For the BD cement, after opening the capsule, 5 drops of liquid were trickled on the material powder. Sequentially, the closed capsule was stirred up for 30 s in an amalgamator (Amalga Mix II, Gnatus, Ribeirão Preto, Brazil). For MTA and EC a powder/liquid ratio of 1:0.33 (g/g) was used; the liquid was gently mixed with the powder previously positioned on a glass plate and, with the help of a #24 spatula, they were mixed until a uniform mass was formed (approximately 30 s). Immediately after manipulation, the materials were placed in the wells and left at 37 °C and 100% humidity during the setting time of the material. The lingual surfaces of the samples were then sealed with composite (A2 Opallis Flow, FGM Dental Group, Joinville, Brazil) and light-cured for 60 seconds. Two additional specimens were prepared and restored with composite only to serve as a control.

After restoration, the specimens were immersed in flasks containing 10 mL of distilled water and placed in an oven at 37 °C, where they remained throughout the experiment. Before immersion, the parameters “L”, “a” and “b” were recorded again (D0 - baseline) to determine the color change (ΔE) during the entire immersion time [15]. The blocks were evaluated after 14, 30, 120 and 150 days by measuring the color parameters as previously described. Additional care was taken for color measurements: The operator was previously intensely calibrated and blinded to the group under analysis; the analyzes were carried out in an environment with controlled and standardized lighting throughout

the experiment; the use of a padronized silicone matrix to standardize the measurement position.

The ΔE values were calculated using the following formula [17]:

$$\Delta E = \left[(Ln - L0)^2 + (an - a0)^2 + (bn - b0)^2 \right]^{1/2} \quad (1)$$

At the end of the test period, a representative specimen from each group was embedded in acrylic resin, analyzed in cross-section, and photographed.

Statistical analysis

The ΔE values were recorded and each assessment period was compared with the D0 values (baseline). These data were tabulated and analyzed using the Shapiro-Wilk test to determine the normality of the data. Once their parametric nature was confirmed, they were analysed using the ANOVA and Tukey test for individual comparisons, with the significance level set at 5% in each case. In addition, the variation in brightness between the initial and final measurements of the experiment (ΔL , 150 days) was measured and the data were treated as described above.

RESULTS

The initial color parameters analysis was carried out after the distribution of the samples among the groups. It confirmed their statistical similarity ($p = 0.91$) and the homogeneity of the sample. The Control group specimens do not present any significant color variation during the analyzed period ($p = 0.88$), attesting the method security.

The ΔE changes induced by the commercial cements (BD and MTA) and the EC are shown in Table II. All tested materials induced tooth discoloration to an extent that is considered

clinically conspicuous [$\Delta E > 3.7$] [15,25]. The White MTA-Angelus exhibited the most significant color change at 14 days, with its color change being statistically greater than that of BD and EC. At 30 days, Biodentine showed the highest values, surpassing both MTA and EC. At 120 and 150 days, the EC samples displayed lower values compared to BD and MTA. Considering the analysis of the behavior of the materials between the analyzed periods, significant differences were observed in both BD and EC ($p < 0.05$). In EC, despite presenting higher values at 30 days, there was a reduction in them to the point of reaching 120/150 with the patterns observed at 14 days. BD, on the other hand, also showed an increase in darkening values at 30, however, even showing some reduction at 120/150 days, the values at the end. The MTA do not presented variation between the periods ($p > 0.05$).

Table II shows the mean and standard deviation of the luminosity of the specimens in each group. In this evaluation, the EC specimens showed the lowest deviation ($\Delta L = 2.7$), which was statistically different from that of the commercial materials ($p < 0.05$). In the samples from the control group, which were only restored with composite, the ΔE values remained within the clinically perceptible limits. Figure 1 shows a picture of representative samples from the test groups.

DISCUSSION

In restorative procedures, various efforts are made to preserve dentin and pulp tissue. Regenerative procedures have been extensively studied to develop clear and safe protocols for situations where preservation of these structures is critical to pulp vitality. These procedures require the use of a material with good physicochemical properties in combination

Table II - Values (Mean and sd) of tooth discoloration (ΔE) and luminosity (L) and its variation caused by the cements evaluated during the experimental period

Material	ΔE								L					
	14 d		30 d		120 d		150 d		Immediately		150 d		Variation	
	Mean	sd	Mean	sd	Mean	sd	Mean	sd	Mean	sd	Mean	sd	Mean	sd
Biodentine	4.52 ^{aA}	2.27	14.49 ^{b,C}	1.61	7.65 ^{b,B}	1.06	7.93 ^{b,B}	2.68	96.9	2.52	88.8	3.9	8.1 ^b	2.9
White MTA-Angelus	8.35 ^{bA}	1.33	8.37 ^{aA}	3.03	8.45 ^{bA}	2.34	9.34 ^{bA}	2.98	99.0	0.76	88.1	0.91	9.9 ^b	1.6
Experimental cement	4.69 ^{aA}	2.91	7.05 ^{aB}	3.2	3.8 ^{aA}	2.64	4.08 ^{aA}	1.8	91.3	4.53	88.7	4.16	2.7 ^a	3.3

^{a,b}Different lower-case superscript letters represent significant differences between the experimental groups in the periods evaluated according to the ANOVA and Tukey tests, both with $p < 0.05$. ^{A,B}Different upper-case superscript letters represent significant differences between the analysed periods in each group according to the ANOVA and Tukey tests, both with $p < 0.05$.

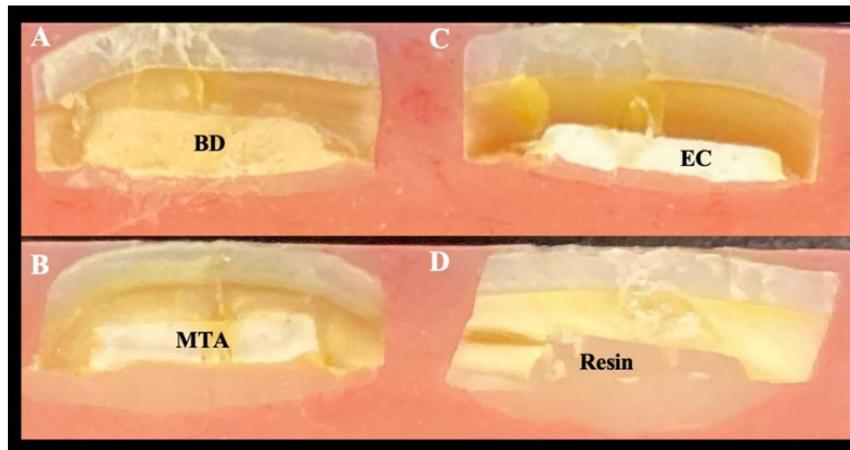


Figure 1 - Representative image of specimens from the groups evaluated after 150 days (A- BD; B- MTA; C- EC; D- Control).

with excellent biological properties [26-28]. Therefore, these materials should be able to induce remineralization in procedures such as pulp capping or revascularization of blood clots.

Furthermore, these materials should not induce tooth discoloration [25]. The aim of the present study was therefore to evaluate the color changes induced by an experimental tricalcium silicate-based material in comparison to those induced by commercially available cements (BD and MTA). The results of this study indicate that all materials caused some degree of color change. However, the EC group caused less discoloration compared to the other cements and remained close to clinically perceptible values (> 3.7). Therefore, the null hypothesis of this study was rejected.

The methods used in this study followed the pattern of previous studies [4,9,24], which were based on an earlier classic study [17]. Bovine teeth were also used in these studies. The use of bovine teeth and human teeth has been investigated previously and showed similar results with both substrates [24]; therefore, the use of bovine teeth is justified for this purpose. The assessment of tooth discoloration using spectrophotometry is another point of convergence between the listed studies, although the assessment time in each study was highly individual. Furthermore, regarding the systematic analysis of color data, two models are most common, CIELab and CIEDE2000. The latter has been attributed greater sensitivity [29] however, studies that used both did not identify discrepancies in results [30-32]; endodontic literature usually uses CIELab [9,19,24], which is why, in the name of comparing results, this model was chosen. The analysis of the change in

lightness (“L”) was added because it could allow a better assessment of tooth darkening.

In general, the values found here are compatible with those available in the literature [9,11,19,24]. An interesting finding concerns their variation, independent of cement, over time. In the present study, the three cements experienced variations in their darkening values, with a peak at 30 days, followed by a reduction. This fact has been attributed to an initial concentration of staining, followed by a kind of spreading, generally softening the measured color parameters [9,19,24].

The color change analysis revealed that the samples from the EC group had significantly lower ΔE values compared to the two commercial cements at 150 days follow-up ($p < 0.05$). Although the values were above the threshold of clinical perceptibility (< 3.7), they were very close to this target (4.08). Considering the periods evaluated, the EC presents, at the end, values similar to those initially presented ($p > 0.05$). In addition, the EC group also showed the least variation in brightness ($P < 0.05$). This behavior was somewhat expected as zirconium oxide is used as a radiopacifier, which is considered safe in terms of tooth discoloration [24].

In contrast, the BD cement caused (150 days) a significant color change despite the use of zirconium oxide as radiopacifier (7.93). As for behavior throughout the evaluation period, it ultimately showed a significant difference compared to the parameters observed at 14 days ($p < 0.05$). In addition, a brightness variation of about 10% was observed in the BD group (8.1). This observation is not new in the literature, as systematic reviews

also report the observation of color changes after the use of BD [33]. This occurrence could be due to the presence of yellow and red pigments in addition to brown iron oxide in the composition specified by the manufacturer [21].

Clinically noticeable color changes also occurred in the MTA group, with values peaking after 150 days [4,25]. Over time, no significant variation was observed ($p > 0.05$). This group also showed the greatest variation in luminosity in this study (9,9). The composition of this material was recently changed, replacing bismuth oxide with calcium tungstate [4]. So, it is difficult to compare the results of the present study with those available in the literature. However, a previous study had already indicated the occurrence of tooth discoloration in materials with calcium tungstate as radiopacifier [34]. Although the new formulation shows color changes, the values are much lower than those of the original formulation using bismuth oxide [19].

In summary, although the EC cements showed lower color changes compared to the commercial cements, the *in vitro* nature of this study limits the extrapolation of the results. Therefore, future studies are needed to evaluate the performance of this new material under conditions more like oral fluids and in the presence of blood clots, as well as *in vivo* experiments.

CONCLUSION

Under the conditions of this study, the experimental tricalcium silicate cement showed less color change compared to the commercially available cements Biodentine and white MTA-Angelus over a 5-month period, suggesting potential applicability for clinical use in aesthetic areas.

Author's Contributions

SMLC, JSM, BCV, MAHD: Conceptualization. SMLC, BAA, AGL: Data curation. JSM, MAHD: Formal Analysis. BCV, JSM: Funding Acquisition. JSM, BCV: Supervision. SMLC, AGL, BCV, JSM Writing – Original Draft Preparation, JSM, BCV, MAHD Writing – Review & Editing.

Conflict of Interest

The authors have no conflicts of interest to declare.

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Regulatory Statement

This study does not require ethics approval.

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Bruno Carvalho de Vasconcelos
(Corresponding address)

Universidade Federal do Ceará, Programa de Pós-graduação em Odontologia,
Fortaleza, CE, Brazil.
Email: bcv@ufc.br

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