



# **Tooth Discoloration Induced by Calcium Silicate-based Sealers**

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## **Authors' contributions**

*This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.*

## **Article Information**

DOI: <https://doi.org/10.9734/jammr/2024/v36i85526>

## **Open Peer Review History:**

This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: <https://www.sdiarticle5.com/review-history/120745>

**Original Research Article**

**Received: 20/05/2024**

**Accepted: 22/07/2024**

**Published: 25/07/2024**

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**Cite as:** Alencar, David Saldanha de Brito, Larissa Carvalho Novaes Batista, Jaqueline Martins Crivelari, Andre Luiz Almeida de Abreu, Anna Carolina Neves Leutz, Arian Braidó, Leandro Ferreira Frade Soares, Bruno Martini Guimarães, and Marina Angélica Marciano. 2024. "Tooth Discoloration Induced by Calcium Silicate-Based Sealers". *Journal of Advances in Medicine and Medical Research* 36 (8):63-71. <https://doi.org/10.9734/jammr/2024/v36i85526>.

## ABSTRACT

**Aims:** The propose of this study was to evaluate, using spectrophotometric analysis, the change in tooth color induced by different calcium silicate-based sealers over the period of 24 hours and 30 days.

**Methodology:** Sixty bovine teeth were sectioned in enamel-dentin blocks. They were filled with AH Plus Bioceramic, AH Plus Jet, Bio-C Sealer and BioRoot RCS and sealed with composite. Triple antibiotic paste and unfilled samples were positive and negative control groups, respectively. Specimens were stored in separate flasks immersed in tap water at 37°C with ambient light block out. Spectrophotometric analysis for color assessment was performed at different periods, before filling, 24 hours, and 30 days after filling. Luminosity (L) and color change ( $\Delta E$ ) were calculated. The statistical analysis was performed by using ANOVA and Tukey's test ( $P < 0.05$ ).

**Results:** All calcium silicate-based sealers exhibited high color alteration after the evaluated period of 30 days and showed statistically significant difference values of  $\Delta E$  in relation with the negative control in the period of 30 days ( $P < 0.05$ ). Luminosity was not affected in comparison with negative control, in both evaluated periods ( $P < 0.05$ ).

**Conclusion:** All tested sealers showed certain levels of tooth discoloration. Calcium silicate-based sealers (AH Plus Bioceramic, Bio-C Sealer, and BioRoot RCS) presented discoloration exceeding acceptable values within a 30-day follow-up. Notwithstanding, these alterations were imperceptible to the human eye.

*Keywords: Silicate Cemente; dental cements; spectrophotometry; tooth discoloration.*

## 1. INTRODUCTION

Calcium silicate-based materials have been presenting interesting clinical outcomes in endodontic treatments due to its adequate bioactivity and biocompatibility [1]. Such procedures, occasionally, implicate the application of these types of endodontic materials in the coronal portion of the tooth, hence regenerative endodontic procedures, pulp capping and perforation treatment [2-5].

The first formulations of calcium silicate-based materials exhibited gray color, which induced color alteration on the tooth structure [6,7]. This perceived disadvantage was expected to be addressed when manufacturers developed a tooth-colored formulation material, referred to as white MTA. This innovative formulation featured a reduction in iron content [7-9]. However, even with this tooth-colored formulation, dental staining occurred after contact with dentine [10-14]. The radiopacifying agent bismuth oxide was verified to be the major responsible for tooth staining [15-17].

Over the years, others calcium silicate-based materials have been introduced to the market as sealers and filling materials for obturation and root repair purposes, incorporating different radiopacifier components, such as zirconium oxide and calcium tungstate [18]. Despite efforts to mitigate tooth staining, color changes still occur, although nearly imperceptible to the

human eye [13,19]. In general, the contact with blood, NaOCl, chlorhexidine or glutaraldehyde can contribute to intensifying dental color alterations [20-22]. Based on these interactions, root canal sealers, which penetrate the dentinal tubules and reach the dental crown are of great relevance and need to be tested for color alteration [16,23-25].

While the literature initially focused on color alterations in root repair calcium silicate-based cements, the diverse compositions of bioceramics materials, incorporating various radiopacifying agents and additives, still requires investigation. This *in vitro* study aimed to evaluate, through spectrophotometry, the changes in tooth color induced by different calcium silicate-based sealers over the period of 24 hours and 30 days. The null hypothesis was that all test sealers would have no significant differences in luminosity and color change ( $\Delta E$ ) values within an acceptable range.

## 2. MATERIALS AND METHODS

### 2.1 Sample Size Calculation

The sample size for bovine teeth was justified based on previous articles [16,26,27]. The sample size was calculated using G\*Power v3.1 program for Mac (Heinrich Heine, University of Dusseldorf) with the comparison test between more than 2 methods with independent groups (ANOVA). From the data obtained and based on 4 research groups, a standard deviation

estimated of 2.67 was used and a minimum difference to be detected in the value of 7.84. Furthermore, a test power of 0.80 ( $\hat{\alpha}$ ) and alpha ( $\hat{\alpha}$ ) of 0.05, resulted in a total of 10 samples ( $n = 10$ ) for each experimental group.

## 2.2 Sample Preparation

The use of human teeth in research is limited by ethical concerns and challenges in obtaining suitable samples. Extracted human teeth often have restorations or caries that can interfere with color analysis. In contrast, bovine teeth provide a flat surface that allows for accurate color assessment and facilitates standardized measurements. The dentin structure of human and bovine teeth is comparable in terms of the number and diameter of tubules. While there may be some variability in the number of tubules, the bovine tooth model has been utilized as a substitute for human teeth in tests due to the similarity of the collagen organic matrix, as both contain collagen type I [16].

A total of 60 bovine mature teeth were selected for this study. They were cleaned, and the crowns were sectioned with a 0.3mm diamond disk (Isomet; Buehler, Lake Bluff, IL), to obtain 10 x 10mm enamel-dentin blocks with thickness standardized at  $3.5 \pm 0.1$ mm confirmed with a vernier. In the center of the dentinal surface, a cavity with 5-mm diameter and 1.5-mm depth was prepared with a diamond bur 4054 (KG Sorensen, São Paulo, SP, Brazil). The samples were immersed in 1% sodium hypochlorite for 30 minutes, to simulate the total time of contact during a root canal treatment. They were then washed with distilled water, washed using 17% EDTA (pH 7.5) for 2 minutes, followed by a final rinse with distilled water [28]. The samples were dried with gauze and then separated into 4 groups ( $n = 10$ ), according to the materials tested: AH Plus Bioceramic (Dentsply Sirona, Charlotte, NC, USA), AH Plus Jet (Dentsply Maillefer, Ballaigues, Switzerland), Bio-C Sealer (Angelus, Londrina, PR, Brazil), BioRoot RCS (Septodont, Saint-Maur-des-Fossés, France).

Each sample had your cavity filled with the sealers tested. Positive (triple antibiotic paste) and negative (left unfilled) control groups were also assembled. All materials evaluated were mixed according to the manufacturer's instructions. Triple antibiotic paste containing metronidazole, minocycline and ciprofloxacin was prepared following a previous study (Trope 1978). The outer limit of each cavity was conditioned with 37% phosphoric acid for 30

seconds, washed with distilled water for 1 minute and then dried with an air syringe. Adhesive (Single Bond Universal; 3M ESPE, Germany) was applied in the outer limit of the cavity and light-cured (Radii-Cal; SDI Limited, Bayswater, Australia) for 20 seconds. The sealers were placed into the prepared cavities at a depth of 1.5mm. Succeeding the materials set, the cavities were sealed with a flow composite resin B2 (Nova DFL, Rio de Janeiro, RJ, Brazil) and light-cured again for 60 seconds. The samples were stored individually in dark flasks and immersed in tap water at 37° C with ambient light blocked out.

## 2.3 Color Assessment of Tooth in Contact with Different Calcium Silicate Based Sealers

The assessment of tooth discoloration was performed once for each period: before filling, 24 hours, and 30 days after filling. A spectrophotometer (Vita Easyshade; VITA Zahnfabrik, Bad Sackingen, Germany) was used for assessments. The samples were retrieved from the flasks and the excess of water was eliminated with a gauze, then placed into an adapted plastic box with a 5mm diameter hole, where the enamel side of the samples were positioned. The box was closed, and the tip of the spectrophotometer was positioned in the hole to measure the color in the same position for every sample.

The color was established following the CIELAB color system, as defined by the International Commission on Illumination (Commission Internationale De L'Eclairage 1978). The values of luminosity ( $L^*$ ) represents the lightness values,  $a^*$  represents the values of red-green and  $b^*$  represents the values of yellow-blue. These values are numeric and use the formula  $\Delta E = [(L_1 - L_0)^2 + (a_1 - a_0)^2 + (b_1 - b_0)^2]^{1/2}$  to achieve an estimated degree of color change ( $\Delta E$ ). The lightness values ( $L$ ) were obtained for all periods directly in the spectrophotometer and were used to evaluate the darkening of the groups.

## 2.4 Representative Stereomicroscopic Analysis of Samples

Randomly selected samples of each one of the sealers tested were sectioned in the center by using a 0.3mm diamond disk (Buehler). Representative images of each sample were taken through a stereomicroscope (M50; Leica Microsystems, Wetzlar, Germany) and the Leica Application Suite software (Leica Microsystems).

## 2.5 Statistical Analysis

GraphPad Prism 9 software (GraphPad Software, San Diego, USA) was used for statistical analysis. For luminosity ( $L^*$ ) and color change ( $\Delta E$ ) analysis, normal distribution was found and confirmed with Shapiro-Wilk test and parametric distributions were assessed using ANOVA and a post-hoc analysis with Tuckey's test at a 5% significance level ( $P < 0.05$ ).

## 3. RESULTS AND DISCUSSION

The values of mean, standard deviation, and statistical differences of the luminosity ( $L^*$ ) and

color change ( $\Delta E$ ) in each period are shown in Fig. 1 and stereomicroscopic representative images presented in Fig. 2. For  $L^*$  values, no statistical differences were found among the test calcium silicate-based sealers in both periods (24 hours and 30 days) ( $P > 0.05$ ). Regarding the  $\Delta E$ , all calcium silicate-based sealers exhibited increased color alteration after the evaluated period of 30 days and showed statistically significant difference values of  $\Delta E$  in relation with the negative control in the period of 30 days ( $P < 0.05$ ). AH Plus Jet did not show statistically significant difference when compared to the negative control ( $P > 0.05$ ).

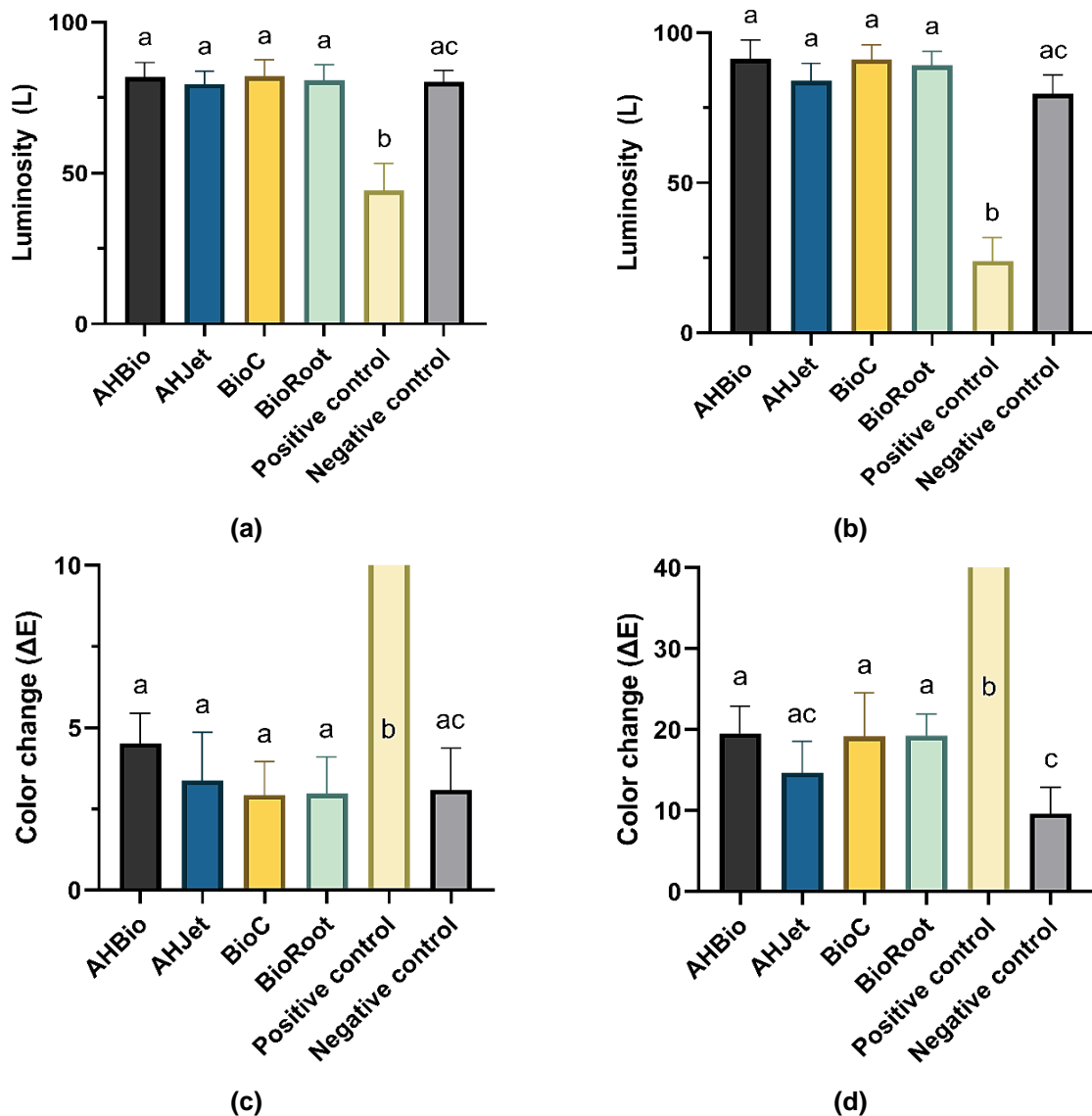
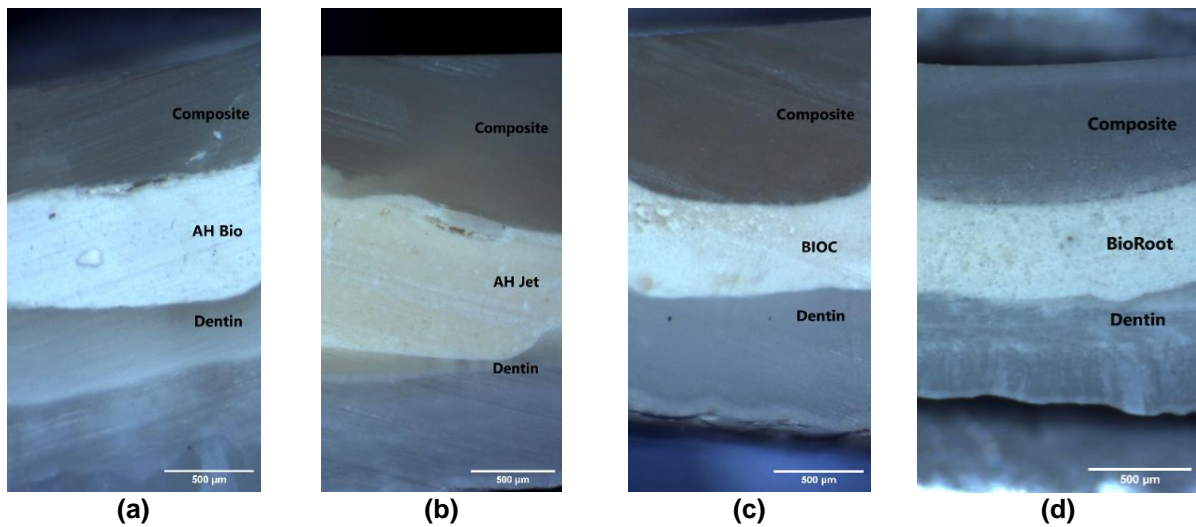


Fig. 1. Mean and standard deviation of luminosity ( $L$ ) at 24 hours (a) and 30 days (b) of analysis and mean and standard deviation of color change ( $\Delta E$ ) at 24 hours (c) and 30 days (d) of analysis. Different lowercase letters indicate statistical differences



**Fig. 2. Representative images of sectioned samples filled with AH Plus Bioceramic (a), AH Plus Jet (b), Bio-C Sealer (c) and BioRoot RCS (d)**

Within the limits of this *in vitro* study, this research aimed to evaluate the change in tooth color induced by different calcium silicate-based sealers over the period of 24 hours and 30 days.

Dental discoloration is an undesirable consequence resulting from chemical reactions between certain substances and the tooth structure. These materials penetrate the dentinal tubules and can reach the dental crown, raising concerns about potential color alterations. Consequently, testing the effects of these sealers on tooth color is crucial for understanding and minimizing aesthetic complications in dental treatments [16].

The primary cause of discoloration resulting from bioceramic sealers is the presence of radiopacifiers like bismuth oxide, which can become destabilized when exposed to strong oxidizing agents such as sodium hypochlorite or amino acids found in dentin collagen. Studies have demonstrated that bismuth oxide may undergo a phase change when in the presence of collagen and strong oxidizing agents, leading to discoloration. To prevent discoloration of white MTA, it has been recommended to explore alternatives such as replacing the radiopacifying agent in the MTA formulation [1,16,17].

Regarding sample selection, bovine teeth were chosen for this spectrophotometric analysis, in accordance with findings from prior research [28,20,29]. The absence of differences between human and bovine substrates in color alteration studies suggests that bovine teeth can be employed as a suitable substitute [30,31].

However, further studies are needed to evaluate the potential impact on result interpretation in experiments utilizing bovine tooth substrates [32].

Spectrophotometry is the gold standard for measuring dental color, it allows the measurement of CIE Lab space color coordinates, which stands for the brightness or luminosity (black and white), the basic colors red and green (a) and yellow and blue (b) [7,28].

In most of *In vitro* studies evaluating color change or discoloration aspects of teeth induced by calcium silicate-based materials are mainly regarding root repair purposes cements, which is correlated with its application in the pulp chamber or in regenerative endodontic procedures [13,14,16,33,34].

Color change assessment concerning calcium silicate-based sealers have only two studies [19,35] at this present moment. These studies investigated tooth discoloration potential in different time stamps, up to 6 months and 3 years respectively, in which human teeth were filled with MTA Fillapex, iRoot SP, BioRoot RCS and Total Fill BC Sealer. Although their results presented  $\Delta E$  measurements above standard values with statistically significant results when compared to the control group, but with no significant difference compared to each other, the Luminosity component were the most influential aspect after 6 months. Parallely, in our study Luminosity was the least affected variable after 1 month, perhaps such divergence was

seen because of the difference in evaluation times.

The dental color alteration induced by sealers in certain levels was described when filling material, such as gutta-percha combined with sealers, was not fully cleaned from the pulp chamber, hence, root canal filling materials are left 2mm below the cemento-enamel junction, since tooth color change seems to occur less at this limit [36,37]. Parallely, though it's not protocol, even in endodontic treatments which apply a backfill above the gutta-percha cut up to the cervical entrance of the root canal preceding the restorative procedures, the risk of tooth discoloration while using calcium silicate-based materials may still occur due to its ability to penetrate and diffuse through the dentinal tubules over time, regardless of the coronal sealing or adhesive material [38]. Although, from a clinical perspective, chemical-mechanical preparation, cleaning and drying of the root canals for the obturation step in endodontic procedures still minimize the discoloration in certain levels [19].

This study evaluated the change in tooth color induced by different calcium silicate-based sealers. Luminosity component was the least affected variable through all experimental groups in both periods evaluated. Interestingly, even though all calcium silicate-based sealers evaluated showed statistically significant difference values of  $\Delta E$  in relation with the negative control in the period of 30 days, that was not a level of discoloration possible to distinguish through the human eye. AH Plus Jet presented the most stable color change between all materials tested after 30 days, which means that both clinical and experimental color change aspects did not differ from each other. Different results were found in slightly similar researches [19,35], which assessed color change in human tooth crowns, and despite all differences between methodologies, management of sample selection and storage, and scientific limits of both studies, it was found higher values of L and  $\Delta E$  related to AH Plus when evaluated at 1 month and maximum discoloration was presented at the period of 6 months.

The time for color alteration to begin appears to vary according to the materials used. Previous literature describes time stamps of 24 hours for MTA [39] and 6 months and one year for Biodentine or non-bismuth oxide cements [40] with maximum darkening appearing at 6 months

without significant variations throughout follow-up periods [19].

The null hypothesis was rejected since the color change values of calcium silicate-based sealers exceeded the acceptability levels. Still, the significant differences in terms of  $\Delta E$  found in both time stamps of this study comparing AH Plus Jet with calcium silicate-based sealers and the controls presents a united front in terms of a clinical point of view on how these materials can affect aesthetic over time, since in general, studies with different application methodologies and follow-up times showed results which did not exceeded acceptability levels or showed differences when compared to control groups [19,35]. However, the differences, usually, are not significant.

Regarding an experimental angle, the relation of calcium silicate-based sealers and the assessment of color change in teeth needs to diverge studies from root repair calcium silicate-based cements, since different applications methods may be a cause of bias.

From clinical perspective, the color change in tooth while using calcium silicate-based sealers are still in tolerable values, since the variation is not noticeable through human eyes, yet further studies need to be carried out to contribute plainly to understanding color change stability over time.

#### 4. CONCLUSION

All tested sealers showed certain levels of tooth discoloration. However, clinically, despite all discoloration values increasing after 30 days, there was no clinical influence because the color change cannot be perceived by the human eye. These results indicate that, although discoloration occurred, it is not clinically relevant from an aesthetic point of view.

#### DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

#### CONSENT AND ETHICAL APPROVAL

It is not applicable.

## ACKNOWLEDGEMENT

The authors are thankful to the Coordination for the Improvement of Higher Education Personnel (CAPES) – Finance Code 001.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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