Heliyon 9 (2023) e13237

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Effect of calcium silicate-based endodontic sealers on tooth color: A 3-year *in vitro* experimental study

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ARTICLE INFO

Keywords:
Calcium silicate-based sealers
Endodontic sealers
Tooth color
Bioceramic sealers

ABSTRACT

Objective: To analyze the change in tooth color produced by two hydraulic and one resin-based sealers by means of spectrophotometry for 3 years.
Methods: Forty maxillary anterior teeth were selected (n = 10 per group). Root canals were prepared by rotary instrumentation and irrigation was performed with NaOCl, which was also used in the final irrigation, followed by saline and activated with Endoactivator. Root canals were then filled using single cone technique. Negative control (NC): gutta-percha filling; Positive control: gutta-percha and AH Plus; experimental groups: gutta-percha and Bioroot RCS/TotalFill BC Sealer. Gutta-percha was cut 2 mm below the cementoenamel junction, the pulp chamber was sealed with flowable composite. The teeth were kept in PBS. Color was measured in the cervical and incisal halves before root canal treatment (RCT), one and six months after RCT, and after one, two and three years, with the Vita EasyShade spectrophotometer; positioned using an individualized splint. ΔE_{ab} and ΔE_{00} were calculated. Two-way ANOVA repeated measures test followed by Bonferroni post-test were performed to analyze the ΔL, Δa*, Δb*, ΔE_{ab}, and ΔE_{00}, considering treatment groups and evaluation periods. Statistical significance was set at $p < 0.05$.
Results: In the incisal half, in decreasing order of darkening, the groups at three years were ordered according to the ΔE_{ab}: AH Plus > NC > TotalFill > Bioroot. ΔE_{00} values were: 1.38 ± 0.61 NC, 2.37 ± 0.70 AH Plus, 1.86 ± 0.60 BioRoot and 1.53 ± 0.85 TotalFill. In the cervical half, the ΔE_{ab} values, showed the same descending order, except for Bioroot and TotalFill which alternated the order. The ΔE_{00} values were 1.86 ± 0.61 NC, 3.01 ± 0.70 AH Plus,1.89 ± 0.58 Bioroot and 1.65 ± 0.41 TotalFill, with no significant differences between groups and times in both locations.
Conclusions: All groups presented some degree of discoloration. Lightness and b* component were the most influential. Calcium silicate-based cements (Bioroot RCS and TotalFill BC Sealer) produced acceptable levels of discoloration at the end of follow-up.

1. Introduction

Tooth discoloration after endodontic treatment is a common esthetic problem, particularly in anterior teeth. In addition to the pathology that caused the need for root canal treatment, the products and materials used are also implicated in discoloration. Certain components of these materials, such as eugenol, bismuth oxide or silver, as well as chemical reactions that occur in contact with

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https://doi.org/10.1016/j.heliyon.2023.e13237
Received 16 September 2022; Received in revised form 13 January 2023; Accepted 23 January 2023
Available online 25 January 2023

irrigants, dentin collagen, or blood, can cause discoloration [1,2].

From a pathogenic point of view, bismuth oxide, used as a radiopacifier in some cements, reacts with dentin collagen causing a grayish discoloration. It also reacts in contact with sodium hypochlorite (NaOCl), giving rise to sodium bismuthate which has a dark brown color. Upon contact with chlorhexidine, blood, or glutaraldehyde, it also produces dark compounds. These products penetrate through the dentinal tubules and can reach the dental crown and cause color change [3,4,5,6,7].

To minimize the problems arising from the discoloration caused by bismuth oxide, alternative radiopacifiers have been proposed since 2002: zirconium oxide, tantalum oxide, calcium tungstate, barium zirconate, or barium sulfate. Their radiopacity is lower compared to bismuth oxide [8]. However, most calcium silicate-based endodontic sealers have a radiopacity between 5 and 8 mm of aluminum, exceeding the minimum radiopacity required for a sealer cement, which is 3 mm of aluminum (3 times more radiopaque than dentin) [9].

Among the scientific literature, dental discoloration has been described with greater intensity with resin-based cements (particularly with AH26), eugenol-based cements, and with mineral trioxide aggregate (MTA) with bismuth oxide as radiopacifier [10,11,12,13,14,15]. With other hydraulic cements, such as Biodentine, Endosequence, Bioaggregate or the new formulation of MTA Angelus containing other types of radiopacifiers, a lower degree of discoloration is described, although most studies refer to their application as a root repair materials or in regenerative endodontic procedures and not as a root canal sealers [7,13,16–18]. In general, all hydraulic cements in contact with blood cause a certain degree of dental discoloration [5,19]. Contact with NaOCl or chlorhexidine also produces a certain degree of discoloration of the cement and of the tooth structure itself [12,20].

Spectrophotometry is the gold standard for measuring dental color [21]. The spectrophotometer allows the measurement of the color coordinates of the CIELab space, which is a color space in which three dimensions of color are evaluated: the brightness, that ranges from 0 (black) to 100 (white); the distance between the basic colors red and green (a); and the distance between yellow and blue (b). The ΔE_{ab} represents the difference between the final color and the basal color of the tooth [10]. The CIE2000 system provides closer tolerance levels of color change than the CIELAB system. In the CIE2000 system, the color difference (ΔE_{00}) is calculated based on the increase in lightness ($\Delta L'$), chroma ($\Delta C'$), and hue ($\Delta H'$). RT is the rotation function that accounts for the interaction between chroma and hue differences in the blue region. S_L, S_c, and S_H are weighting functions used to adjust the total ΔE_{00} for variation in perceived magnitude with variation in the location of the color coordinate difference between two color readings. Lastly, k_L, k_c, and k_H are the correction terms for the experimental conditions [22,23].

Tolerance refers to two aspects: on the one hand, 50:50 perceptibility, which is the color difference that 50% of the people can perceive and on the other hand, 50:50 acceptability, which refers to the color difference that 50% of the people consider tolerable. A value of no more than 1.8 units is considered acceptable for ΔE_{00} [24].

In the present *in vitro* study, the aim is to analyze the change in tooth color produced by two calcium silicate-based sealers and a resin-based sealer, evaluated by spectrophotometry, over a period of 3 years. The null hypothesis is that all groups will maintain ΔE_{00} values within the level of acceptability.

2. Materials and methods

The protocol for this study was approved by the Ethics Committee of Universitat de València with reference number H152078413452. Tooth donors signed a prior informed consent form.

2.1. Sample selection and preparation

Human single-rooted teeth from the anterior sector with a post-extraction time of no more than one year, extracted for periodontal reasons, were used. The age of the donors ranged between 50 and 70 years old. All teeth were visualized at 10× magnification, and two radiographs were taken to confirm the radicular status. Teeth with caries, restorations, structural alterations (cracks, fractures, fissures), developmental anomalies, calcifications, staining, structural color alterations, or previous root canal treatment were excluded. A sample of 40 teeth free of any of the above exclusion criteria was obtained. The teeth were kept in 0.1% thymol for 48 h and externally cleaned of organic debris, stains or calculus and then placed in Phosphate Buffer Solution (PBS) s for preservation in individual Eppendorf tubes for each specimen.

2.2. Root canal treatment

The biomechanical preparation of the root canals was performed under magnification with an OPMI pico surgical microscope (Carl Zeiss Meditec AG, Germany). All the teeth were instrumented with Protaper Next System (Dentsply Sirona, New York, USA). During

Table 1
Materials and groups.

Groups	Material	Manufacturer	Lot
Negative control (NC)	Guta-percha	Dentsply Sirona.New York, EEUU	1709000158
Positive control (PC)	Guta-percha + AH Plus	Dentsply Sirona.New York, EEUU	1709000157
TotalFill BC Sealer (TF)	Guta-percha + TF	FKG Dentaire SA, La Chaux de fonds, Swiss	17002SP
Bioroot RCS(BR)	Guta-percha + BR	Septodont, Saint-Maur-des-Fossés, Francia	B20365

preparation, 6% NaOCl (Canalpro, Coltène Iberia SL, Majadahonda, Spain) was used as an irrigant. As final irrigation, NaOCl was used, followed by 3 ml of 0.9% (w/v) saline, 17% EDTA (Canalpro, Coltène Iberia SL, Majadahonda, Spain) and saline. Final irrigation was performed with activation using Endoactivator (Dentsply Sirona, New York, USA).

Once instrumented, the teeth were randomly divided into four study groups ($n_i = 10$), [1,3,5], according to the filling material used, as shown in Table 1. In all cases, the single cone technique was used for root canal filling together with and endodontic sealer. In the negative control (NC), only a gutta-percha cone was used. Using heat, the gutta-percha and sealer were removed up to 2 mm below the cementoenamel junction. The access cavities were then cleaned with alcohol and a flowable composite resin was placed to fill the coronal 2 mm of the canal and pulp chamber (Aura Easyflow AE1 (SDI, Victoria, Australia)), equivalent to the A1 shade of the Vita guide, preceded by a total etching technique. The access cavity was finally sealed with a temporary cement (Cavit, 3 M Espe, St. Paul, USA). Teeth were kept at 4 °C for the entire follow-up time in individualized Eppendorf tubes with PBS that was changed monthly.

2.3. Color determination

To evaluate the color at the different times of the study in the same position, each group was mounted on a white silicone positioning base. A 1 mm-thick thermoplastic splint with two perforations for each tooth of 5 mm in diameter was made with a circular scalpel to measure the color in the coronal-cervical portion and in the coronal-incisal portion of the buccal side of the crown. To measure the color, the Vita Easyshade spectrophotometer (Vita Zank Germany) was used, and the CIELab space values (L, a*, b*) were recorded. Each measurement was repeated 3 times and the mean value was calculated. The device was recalibrated after measuring 5 teeth. All measurements were performed by the same operator. The color was measured before the access cavity of the tooth, after the access cavity was performed, after 1 and 6 months after root canal filling, and at 1, 2 and 3 years after root canal filling.

2.4. Data analysis

The L a* b* values at each time of the study were recorded in an Excel sheet for analysis. The values and standard deviations of lightness, ΔL, Δa or red-green distance, and Δb or yellow-blue distance were calculated for each group and time of the study. The three variables were related by calculating the ΔE_{ab} (CIELab), according to the formula:

$$\Delta E_{ab} = ((L_a - L_b)^2 + (a_a - a_b)^2 + (b_a - b^*_b)^2)^{1/2}.$$

The ΔE_{00} (CIEDE2000) was also calculated, according to the formula:

$$\Delta E_{00} = [(\Delta L'/k_L S_L)^2 + (\Delta C'/k_c S_c)^2 + (\Delta H'/k_H S_H)^2 + R_T(\Delta C'/k_c S_c)(\Delta H'/k_H S_H)]^{1/2}$$

All calculations were performed for both the incisal and cervical halves of the teeth. The SPSS 28.0 statistical package (IBM, Chicago, USA) was used. A general linear repeated measures model test followed by Bonferroni posttest were carried out to analyze the ΔL, Δa*, Δb*, ΔE_{ab}, and ΔE_{00}, considering treatment groups and evaluation periods. The equality of the variance-covariance matrix

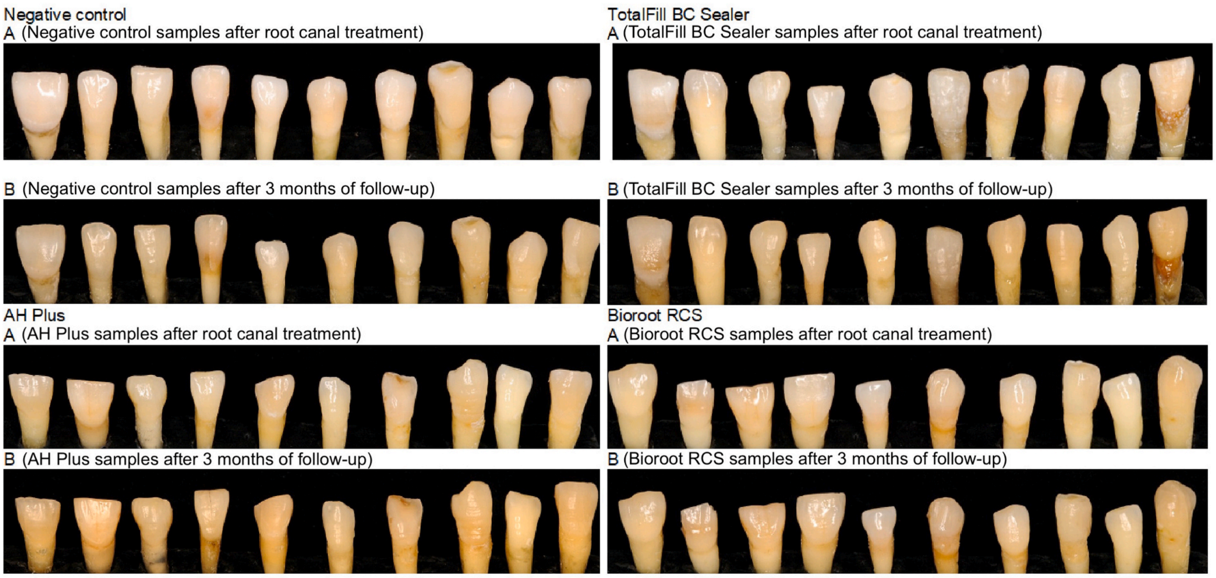

Fig. 1. Clinical images of the study groups' samples. Foot note: Two panels are presented for each of the study groups (negative control, AH Plus, Totalfill BC Sealer, and Bioroot). Panel A shows the samples immediately after root canal treatment. Panel B shows the samples three years after root canal treatment (3 years of follow up).

was determined using Mauchly's test of sphericity. Differences were considered significant when $p < 0.05$.

3. Results

Fig. 1 shows the four study groups samples after completion of root canal treatment and after the three years of follow-up.

3.1. Incisal measurement

There was a reduction in brightness in all the groups and color measurements. Significant differences were found in the AH Plus group compared to the NC at six months ($p = 0.03$). Within each group, a significantly greater reduction in brightness was found in the AH Plus group relative to all other measurements at six months ($p < 0.05$). In the Bioroot and TotalFill groups, the reduction in brightness was significantly higher at 6 months compared to the measurements at 2 and 3 years ($p < 0.05$) (Fig. 2A).

In all groups and visits an approach to green was observed. The increase in a* (red/green distance), did not show significant differences within each group throughout the study, nor between groups in the different color measurements ($p > 0.05$) (Fig. 2B).

The b* values (yellow-blue distance) behaved differently depending on the material. In the NC group, a reduction of the b* component (tendency towards blue) was found in all color measurements, although without significant differences between measurements ($p > 0.05$). In the AH Plus group, an increase of the b* component (tendency towards yellow) was found, being significantly higher at one year of follow-up compared to the first month and the second year ($p < 0.05$). In the Bioroot and Totalfill groups, the changes were limited in all measurements, without significant differences. In the 6-month measurement, significant differences were found between the NC and the rest of the groups ($p < 0.05$) (Fig. 2C).

Regarding the ΔE_{ab}, Fig. 3A shows the graphical representation of the results. It can be seen that at six months the highest values of ΔE_{ab} (maximum darkening) were found, although without significant differences between groups or between the different time points of the study. In decreasing order of darkening, according to the ΔE_{ab} values, the groups were ordered as follows at the end of the follow-up period: AH Plus > NC > TotalFill > Bioroot, ranging from 4.45 ± 0.61 for TotalFill to 6.51 ± 0.11 for AH Plus. Values were calculated in the CIE2000 space, by calculating the ΔE_{00}. At three years the values were: 1.38 ± 0.61 for the NC, 2.37 ± 0.70 for AH Plus, 1.86 ± 0.60 for BioRoot and 1.53 ± 0.85 for TotalFill; with no significant differences between groups (Fig. 3B).

3.2. Cervical half

A reduction in brightness was found in all groups and color measurements. At the 6-month measurement, the AH Plus group showed a significant reduction in brightness compared to the NC and TotalFill ($p < 0.05$). In the AH Plus group, a significantly greater reduction in brightness was exhibited at six months compared to the rest of the measurements ($p < 0.05$) (Fig. 4A).

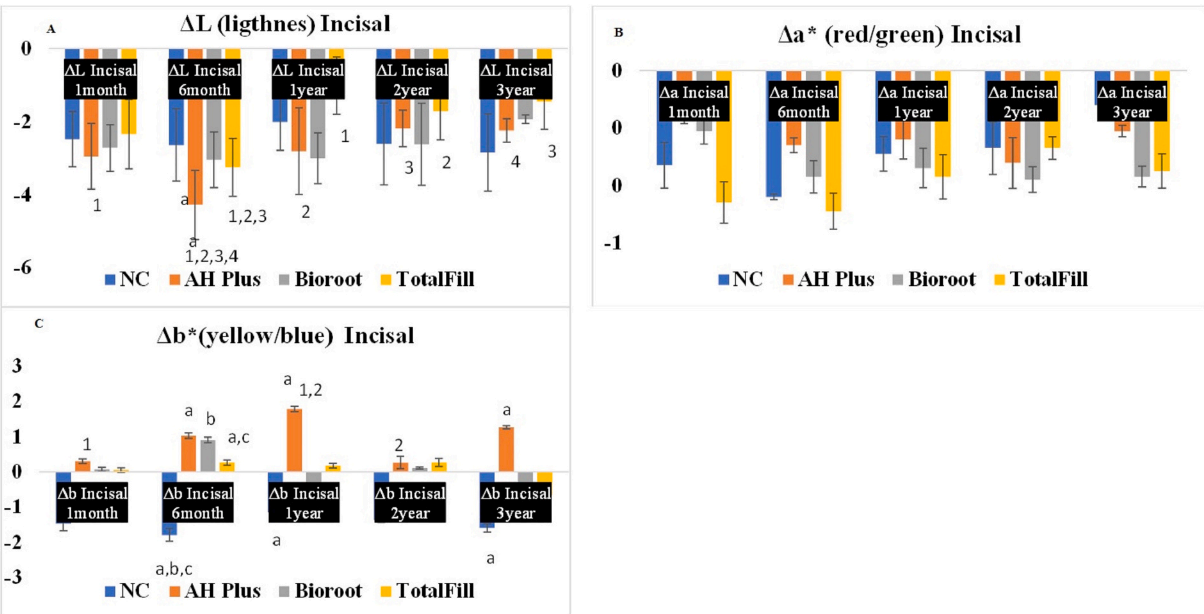

Fig. 2. Mean values and standard deviation of ΔL Lightness (A), Δa red/green distance (B), Δb yellow/blue distance (C) values in the incisal portion. Foot note: The same number within each group indicates significant differences between the different color measurements throughout the study. The same letter indicates significant differences between groups in each color measurement. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

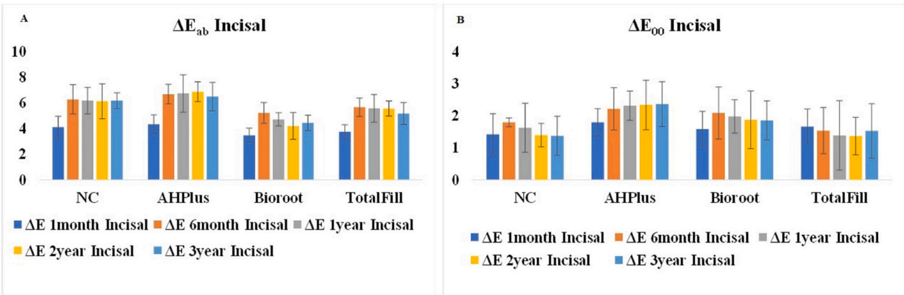

Fig. 3. Mean values and standard deviation of ΔE_{ab} (A) and ΔE_{00} (B) values in the incisal portion.

In all groups and measurements an approach to green was found. The increase in a* (red/green distance) did not show significant differences within each group throughout the study, nor between groups at the different measurements ($p > 0.05$) (Fig. 4B).

The parameter b* (yellow/blue distance) showed an approach to yellow in all groups, with no significant differences within each group for the different color measurements nor within each measurement between groups -$p>0.05$- (Fig. 4C).

Regarding the ΔE_{ab}, the maximum darkening was found at six months in all groups, remaining without significant variations throughout the follow-up period ($p > 0.05$); values are represented in Fig. 5A. In decreasing order of darkening, based on the ΔE_{ab}, the groups were ordered as follows AH Plus > CN > Bioroot > TotalFill. The values ranged from 5.31 ± 1.34 for Bioroot to 8.63 ± 0.98 for AH Plus. At the end of the study, a ΔE_{00} value of 1.86 ± 0.61 was found for the NC, 3.01 ± 0.70 for the AH Plus group, 1.89 ± 0.58 for Bioroot and 1.65 ± 0.41 for TotalFill, with no significant differences between groups (Fig. 5B).

4. Discussion

To our knowledge, there are no *in vitro* studies with a follow-up period of three years in which the change in tooth color of products based on calcium silicate used specifically as root canal sealer was evaluated. In addition, there are no studies that have evaluated the effect on tooth color of the two hydraulic cements assessed in the present work (Bioroot RCS and TotalFill BC Sealer). This can be considered as a strength of our study. However, other materials with this function have been evaluated, some of them based on tri-calcium silicate in shorter follow-up times [1].

In the present study, we used human teeth from different people aged between 50 and 70 years. The age of the patient, the structural changes in the enamel and dentine and the effect of the restorative materials themselves can modify the light transmission.

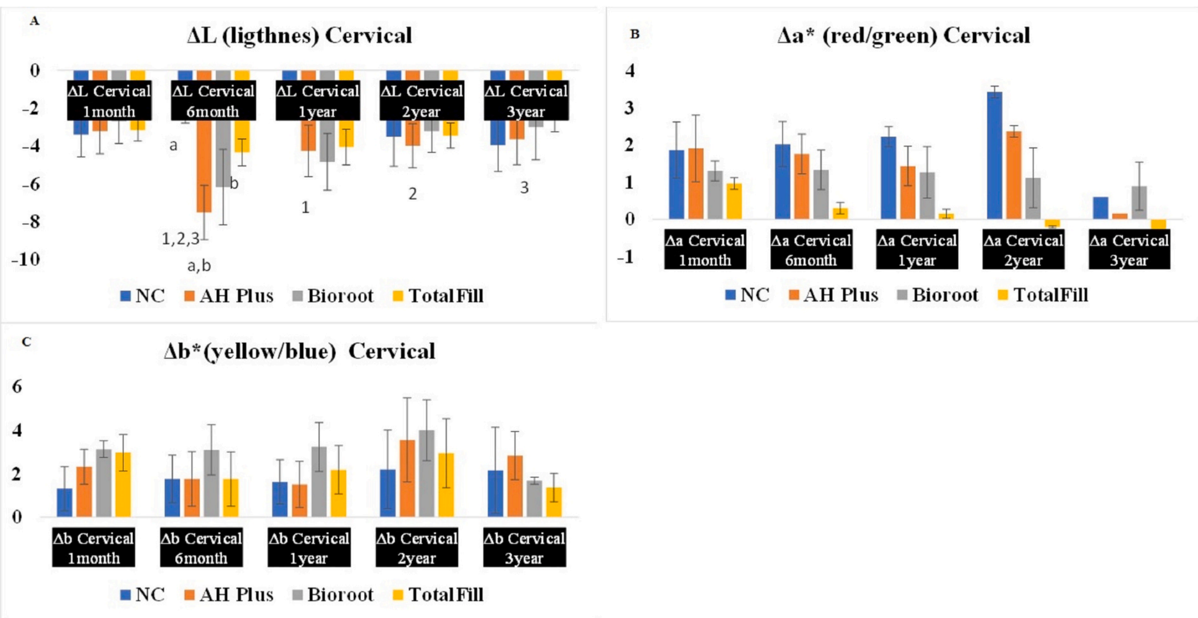

Fig. 4. Mean values and standard deviation of ΔL Lightness (A), Δa red/green distance (B), Δb yellow/blue distance (C) values in the cervical portion. Foot note: The same number within each group indicates significant differences between the different color measurements throughout the study. The same letter indicates significant differences between groups in each color measurement. . (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

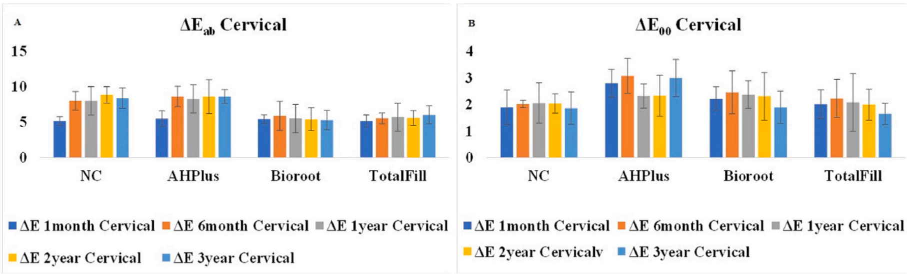

Fig. 5. Mean values and standard deviation of ΔE_{ab} (A) and ΔE_{00} (B) values in the cervical portion.

As recommended among the literature, anterior teeth were used, and radiographs were taken to rule out calcifications, fractures or previous root canal treatment [25]. The impossibility of achieving a completely uniform sample is one of the limitations of studies with human teeth. Random allocation of teeth to study groups may help to reduce this bias.

Although the teeth were cleaned using 0.1% thymol, which has an antioxidant action, they were only kept for 48 h for disinfection. Subsequently, after thorough rinsing with water, they were kept in PBS [26].

In most of the *in vitro* studies in which the change in tooth color produced by tricalcium silicate-based cements was evaluated, the material was placed in the pulp chamber or simulating conditions of regenerative endodontic techniques; showing that the change in color may vary depending on the application method of the material, tooth preparation procedure, disinfection method, irrigant used, and final irrigation sequence [27].

Regarding the use of irrigants, the effect of NaOCl on tooth color has been described in contact with tricalcium silicate-based cements. This is due to the fact that it is able to penetrate between 77 and 300 μm into the dentinal tubules and it is difficult to eliminate completely. When irrigation with saline solution or distilled water is performed after NaOCl, tooth discoloration is lower than when NaOCl alone is used [28]. In the present study, after instrumentation and final irrigation with NaOCl, the canal was flushed with 3 ml of saline, which was activated by Endoactivator system to facilitate its removal from the dentinal tubules and to minimize the effect of the interaction of the irrigant with the root canal sealer.

Parallelly, it has been described that the presence of bismuth oxide as a radiopacifier considerably increases the discoloration effect produced by calcium silicate-based sealers when in contact with NaOCl [4]. However, the tested calcium silicate-based sealers do not have this radiopacifier in their composition.

Another aspect that has been related to dental discoloration is the level at which the gutta-percha is removed and the cleaning of the pulp chamber after the application of the canal filling. It has been demonstrated that when the root canal filling materials are left 2 mm below the cementoenamel junction, tooth discoloration is lower than if it is placed at the limit of the dental cervix [29]. In the present study, both the gutta-percha and root canal sealers were removed up to 2 mm below the cementoenamel junction and the opening cavities were cleaned with alcohol, filling this space and the pulp chamber with an A1 flowable composite preceded by a total etching technique. As described in the scientific literature, sealing the pulp chamber with an adhesive system does not completely eliminate the risk of tooth discoloration when using hydraulic cements for regenerative purposes inside the tooth [30]. In our study, neither the sealer was placed in contact with blood, nor was it used for regenerative purposes. However, the ability of the pigments to penetrate and diffuse, over time, through the dentinal tubules, may justify that some level of discoloration occurred with all the materials studied, despite having performed coronal sealing with an adhesive material and system.

The time after which discoloration begins to appear varies according to the studies and the materials used, ranging from one day for products based on MTA [10] to between 6 months and one year for Biodentine or other hydraulic cements that do not contain bismuth oxide as a radiopacifier [31]. In the present study, color was evaluated after one month of placement, at 6 months, and annually until a period of three years was completed.

The null hypothesis was rejected, since the ΔE_{00} values in the AH Plus group exceeded acceptability levels ($\geq$1.8). On the other hand, no significant differences in terms of ΔE_{ab} or ΔE_{00} were found between the different time points of the study for any of the tested sealers. A study comparing AH Plus with IRoot, a material with the same composition as TotalFill BC Sealer, over a 6-month follow-up period found similar results [32]. Maximum discoloration was found at around 6 months and remained at similar values throughout the remainder of the follow-up period.

The AH Plus group in both the incisal and cervical portions reached a ΔE_{00} value above the tolerance value at the end of the follow-up period. A study in which the ΔE_{00} was used as a measure of color change after the use of different sealers, one of which was AH Plus, also found values higher than those considered acceptable. Values were higher when the limit of the root canal filling was left at the level of the cementoenamel junction, compared to the cases in which it was left 2 mm below [29] A correct obturation level minimizes the penetration of the chemical products that cause discoloration through the dentinal tubules in the dental cervix [11]. This is especially relevant in the anterior sector where, in addition, the thickness of the dentin is smaller [33]. Therefore, from a clinical point of view, especially in the anterior sector, the limit of the filling and the correct sealing of that area is important to minimize the discoloration effect produced by root canal sealers; in addition to the structural changes that occur in the dentin after root canal treatment [33].

In the present study, a maximum discoloration was found at 6 months in the AH Plus group, mainly at the expense of brightness and the b* component (tendency to yellow) with a significant reduction in the later measurements. In the cervical portion, an increase in a* (red tendency) was also found. These data are consistent with those reported in various studies [34,35], but other authors report a progressive discoloration at one year follow-up [36].

The two tricalcium silicate-based cements and the NC group also showed a reduction in brightness, which was maximum at 6 months and decreased in subsequent measurements, both in the incisal and cervical portions. The b* component showed a greater tendency to yellow in the cervical portion, which remained at similar values throughout the follow-up period. The ΔE_{00} at the end of the study was maintained in these groups within the limits of acceptability ($\leq$1.8 units) in both the incisal and cervical portions. In various *in vitro* studies using tricalcium silicate-based cements with different application methodologies and follow-up times, the level of discoloration did not exceed acceptability levels or show differences with compared to controls, except those containing bismuth oxide or when placed in direct contact with blood [3,16,19,31,35,37–42]. However, other authors show discoloration levels above tolerable values in different follow-up periods and simulating different therapeutic conditions [10–12,32–35,43]. However, in general terms, hydraulic cements that do not contain bismuth oxide in their composition show lower levels of discoloration than those containing it, similar to the different control groups used, and lower than resin-based sealers such as AH Plus. However, the differences are generally not significant.

From a clinical point of view, irrigation with physiological saline after the chemical-mechanical debridement procedure of the root canal, the root canal filling level 2 mm below the cementoenamel junction and a correct sealing of the pulp chamber, contribute positively to minimize the discoloration that occurs in the tooth in the medium term after root canal treatment.

5. Conclusion

All the tested sealers caused some degree of discoloration. Brightness and the b* component, in CIELAB space, were the most affected variables. Calcium silicate-based sealers (Bioroot RCS and TotalFill BC Sealer) produced levels of discoloration within the acceptable range after three years of follow-up.

Author contribution statement

Carmen Llena MD. DDS, PhD: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Ana Herrero DDS; Sandra Lloret DDS; Martha Barraza DDS: Performed the experiments; Wrote the paper.

Jose Luis Sanz DDS, PhD: Conceived and designed the experiments; Wrote the paper.

Funding statement

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Data availability statement

Data will be made available on request.

Declaration of interest's statement

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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