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Tooth discoloration caused by endodontic treatment: A cross sectional study

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Abstract
Introduction: This cross-sectional study evaluated the influence of endodontic filling material, cervical limit of root filling, and tooth location on the color variation (ΔE00)from 1 to 60 months of follow-up.
Materials and Methods: Color records were obtained from 70 participant's who received 83 endodontic treatments. CIEL*a*b* color coordinates were measured on the homologous tooth, which was considered as baseline, and on endodontic treated teeth with a spectrophotometer. ΔE00 was calculated by the CIEDE2000 method at three conditions (condition 1: homologous tooth vs endodontically treated tooth after≤1 month; condition 2: homologous tooth vs endodontically treated tooth after >1–-12 months; condition 3: homologous tooth vs endodontically treated tooth after>12 months up to 5 years) for each variable. The zinc oxide and eugenol mineral trioxide aggregate or resin-based filling materials, dental cervix or ≥2 mm in the apical direction cervical limit of root filling and anterior or posterior tooth location were considered in the comparisons. Confidence intervals for the means (95% CI) were calculated,ΔE00 values and CIEL*a*b* individual color coordinates were compared for each pair of variables using the Student t test or Welch test ($\alpha = .05$).
Results: Greater ΔE00 values were generally observed in the condition 3 for cervical limit (ΔE00 = 10.7), use of zinc oxide and eugenol and mineral trioxide aggregate based filling materials (ΔE00 = 10.7), and anterior teeth (ΔE00 = 12.4). Only in the condition1, the ΔE00 values of the filling materials did not show statistical differences ($P = .198$).
Conclusions: Higher ΔE00 values were yielded from zinc oxide and eugenol and mineral trioxide aggregate-based filling materials, anterior teeth, and dental cervix cervical limit of root filling.
Clinical significance: Tooth discoloration yielded by endodontic materials and procedures is a challenge to clinical practice resulting in aesthetic problems and discomfort to both patient and professional, especially when it occurs in anterior teeth.

Keywords: color, endodontic sealers, root canal filling materials, root canal obturation, spectrophotometry, tooth discoloration

Introduction
The literature has shown that biological factors such as disintegration of necrotic pulp tissue and hemorrhage into the pulp chamber may be related to tooth discoloration [1], as well as endodontic materials and procedures [2-5]. Even so, the role played by each pointed factor remains controversial. For instance, the influence of these factors on color variation (ΔE00) [1] may be associated with intra- and/or post-endodontic procedural errors [2,4-6]. Also, nonendodontic etiological factors [7], including idiopathic, metabolic, and traumatic causes, have been proposed as suitable to increase or reduce ΔE00.
Since color is an important property in esthetic dentistry [8-10], especially in anterior teeth, avoiding discoloration after endodontic treatment has become a clinical challenge. Considerable time and money are invested in the attempt to improve the appearance of dis-colored teeth [11]. Advances in techniques and composition of endodontic materials have been the forefront of endodontic research. Nonetheless, despite continuous improvements, tooth discoloration is still considered an undesirable consequence following endodontic treatment [12]. The influence of the material composition on ΔE00 has been reported, considering zinc oxide and eugenol (ZOE)-based [13, 14], mineral trioxide aggregate (MTA)-based [15-18] and

epoxy resin-based sealers, such as AH Plus (Dentsply Sirona, York, Pensilvânia, EUA) [3, 19-20]. These materials usually cause tooth discoloration due to unreacted components or the corrosion of some components owing to moisture and/or chemical interaction with dentin [2, 17-21]. Nonetheless, some procedures as inadequate access cavity may also complicate the clinician's ability to remove root canal filling materials from the pulp chamber while completing the root filling [23-24].
Even though several *in vitro* studies have investigated tooth discoloration related to intra- and/or post-endodontic procedural factors (eg, root canal irrigants, intracanal medicaments, endodontic filling materials, metallic posts and restorations, improper selection/application of tooth-colored restorations) [3, 11, 12, 25], there is still a lack of clinical investigations considering the role of these factors on tooth discoloration. The discrepancies in the *in vitro* models used in these studies make data interpretation difficult, and often the tooth color change is underestimated or overestimated [26]. A better understanding of the factors associated with different degrees of tooth discoloration may provide guidelines for the plan- ning of early interventions that may prevent or reduce this clinical problem [27].
Given the aforementioned, this cross-sectional study evaluated the influence of endodontic filling material, cervical limit of root filling and tooth location on the color variation from 1 to 60 months of follow-up after endodontic treatment based on spectrophotometric analysis. It was hypothesized that the optical properties of endodontically treated tooth could be influenced by the factors under evaluation.

Materials and Methods
This cross-sectional study evaluated the color variation ($\Delta E00$), and CIEL*a*b* coordinates (L*, a*, and b*) of endodontically treated teeth in three conditions (baseline, homologous tooth vs endodontically treated tooth after ≤1 months; baseline, homologous tooth vs end- odontically treated tooth after >1-12 months; baseline, homologous tooth vs endodontically treated tooth after >12 months up to 5 years). The following variables were considered in the comparisons: filling material (AH Plus [Dentsply Sirona York, Pensilvânia, EUA]; Endofill [Dentsply Sirona, York, Pensilvânia, EUA]; Fillcanal [Technew, Rio de Janeiro, Rio de Janeiro, Brazil]; MTA Fillapex [Angelus, Londrina, Par- aná, Brazil]), cervical limit of root filling (dental cervix or ≥2 mm in the apical direction), and tooth location (anterior or posterior teeth). In total, 83 endodontic treatments from 70 participants were evaluated.
The response variables included the color variation ($\Delta E00$), estimated by the CIEDE2000 color difference metric29 and CIEL*a*b* color coordinates obtained from readings made on the homologous tooth (considered as “baseline”) and the endodontically treated tooth (con- sidered as “after”) with a spectrophotometer (Easyshade; Vita Zahnfabrik, Bad Säckingen, Baden-Württemberg, Germany). This study was approved by the Research and Ethics Committee, regis- tered at ClinicalTrials.gov and reported following the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement [29].
All individuals who came to a private dental clinic between January 2019 and November 2021 and who received endodontic treatment and met inclusion criteria were invited to participate. The following inclusion criteria were used to evaluate and enroll potential participants: individuals aged at least 18 years old; endodontic treatment in anterior or posterior (premolar) tooth; homologous tooth without endodontic treatment and/or intraradicular post; at least one-third of crown structure; no amalgam restoration; no artificial crown that would make dental color assessment impossible. Besides,incorrectly filled dental records were also excluded, for example, lack of data regarding the day, month, and year of root canal obturation, filling material used, and periapical radiograph, obtained with the use of radiographic positioner, preserved and with a clear image. All individuals who met inclusion criteria and agreed to participate in the site of the readings. Color difference was estimated by calculating the CIEDE2000 color variation (ΔE_{00}) between each condition, according to the following equation [28]:

Clinical evaluation: Time of the endodontic treatment (day, month, and year); time of data collection (≤1 month, >1-12 months, and >12 months up to 5 years); endodontic material used (resinous sealer: AH Plus/ZOE-based sealer: Endofill and Fillcanal/MTA-based sealer: MTA Fillapex); diagnosis (irreversible pulpitis or pulpal necrosis); and tooth location (anterior or posterior teeth).

Radiographic evaluation: Evaluation of the cervical limit of the root filling [(approximately at the dental cervix, delimited by cemento- enamel junction in the periapical radiographs) or (≥2 mm in the apical direction from the dental cervix)]. For radiographic assessment, standardized intraoral periapical radiographs were obtained at reexamination. All radiographs were taken using the long-cone paralleling technique and a plastic X-ray film holder (Kodak E-speed film, East- man Kodak Co., Rochester, Nova York, EUA) with a standardized exposure time of 0.8 s. The images were photographed in a perpen- dicular incidence against a negatoscope with a digital camera (Canon Rebel T5i DSLR and Canon EF 100 mm f/2.8 Macro USM Lens, Canon Inc., Ota, Tóquio, Jap~ao) for subsequent digital measurements (ImageJ, National Institutes of Health, Bethesda, Maryland, EUA).[30] In order to avoid misinterpretation, all images were viewed on the same monitor in a dimly lit room, under the same conditions, by one calibrated examiner. Radiographic measurements in random endodontic treatments were repeated until the examiner presented high intra examiner reliability as measured by Cohen's Kappa (K = 0.89). A computer-assisted calibration was performed for each radiograph by scale setting assisted by the known data, using TurboReg plugin within the ImageJ (National Institutes of Health, Bethesda, Maryland, EUA) toolkit, providing an increase in the reliability and the precision for the radiographic measurements.
CIEL*a*b* color coordinates evaluation: The CIEL*a*b* color coordinates of the homologous tooth versus endodontically treated tooth at different periods (≤1; >1-12; >12 months up to 5 years after treat- ment) were assessed with a spectrophotometer (Easyshade; Vita Zahnfabrik, Bad Säckingen, Baden-Württemberg, Germany). These homologous teeth, without endodontic treatment, served as a control to test the ability of coronal dentin and enamel to transmit the color induced by the endodontic materials.[4] The spectrophotometer was plugged into a voltage stabilizer to avoid changes in light source intensity. The equipment was calibrated before each reading, and a single trained investigator (JB) made all color measurements, in the same ambient light condition [8]. The active tip of the spectrophotometer was placed at the cervical third of the coronal labial surface of each tooth [10] after previous prophylaxis. For this, a silicone mold was pre- pared and used as a guide for each measurement to standardize the where

$\Delta L0$, $\Delta C0$, and $\Delta H0$ are the differences in lightness, chroma, and hue between two sets of color coordinates; RT is the rotation function that accounts for the interaction between chroma and hue differences in the blue region; SL, SC, and SH are weighting functions used to adjust the total $\Delta E00$ for variation in perceived magnitude with variation in the location of the color coordinate difference between two color readings; and kL, kC, and kH are the correction terms for the experimental conditions. $\Delta E00 \geq 1.8$ is the acceptable color difference threshold for the CIEDE2000 method [9].

For the three conditions, $\Delta E00$ and CIEL*a*b* individual color coordinate values were analyzed considering each pair of variables using the Student t test for equal variance data; the Welch test for unequal variances ($\alpha = .05$). Additionally, 95% confidence intervals (CIs) were calculated.

Results

In total, 83 endodontically treated teeth from 70 participants were included. Twenty- two men (31.43%) and 48 women (68.57%) aged between 18 and 52 years old were evaluated. The following pulp diagnostics were found: irreversible pulpitis in 2 (2.41%), pulpal necrosis in 74 (89.16%), and lack of information in 7 (8.43%) individuals. Regarding periapical status, periapical lesion was observed in 42 (50.60%), no periapical lesion in 29 (34.94%), and periodontal ligament thickening in 12 (14.46%) individuals.

Mean, SD, and CI for $\Delta E00$ in the three conditions are shown in Table 1. All conditions presented $\Delta E00$ values above 1.8, which is the acceptable threshold ($\Delta E00$) for the CIEDE2000 method. Statistically significant difference was found only for cervical limit ($P = .022$) and tooth location ($P = .037$); while no statistically significant difference was observed for endodontic material ($P > .05$) in the first condition (baseline vs ≤1 month). Concerning the $\Delta E00$ obtained in the second condition (baseline vs >1–12 months), statistical significant differ- ences were observed for cervical limit ($P = .037$), endodontic filling material ($P = .050$), and tooth location ($P = .005$). In the third condition (baseline vs >12 months), cervical limit ($P = .000$), endodontic filling material ($P = .000$), and tooth location ($P = .041$) also showed a statistically significant difference. Greater $\Delta E00$ values were observed in the condition 3 for cervical limit at the dental cervix, ZOE, and MTA- based filling materials and anterior location; see Table 1.

For the CIEL*a*b* color coordinates, statistical significant differences were found in all condition and variables evaluated. Decreased L* values were observed for limit at the cervical level in the condition 3 when compared with values obtained for cervical limit at 2 mm in the apical direction ($P < .001$). The lowest L* values were found for ZOE and MTA-based filling materials in the condition 2 (baseline vs >1-12 months) and cervical limit ($P < .041$). Only positive values were obtained for a* and b* coordinates. The b* coordinate show statistically significant difference for location after endodontic treatment in the condition 1 ($P < .014$). In the condition 2, significant a* coordinates color variation was found for location ($P = .046$); while a* and b* coordinates yielded tooth discoloration for variables location ($P = .045$ and $P = .015$, respectively) and filling material ($P = .014$ and $P = .019$, respectively) in condition 3.

Discussion

This cross-sectional study evaluated the influence of endodontic filling material, cervical limit of root filling, and tooth location in tooth discol- oration yielded from 1 to 60 months after endodontic treatment. Since homologous teeth had the same eruption chronology, both had lifelong contact with the same type of dyes, and the maturation occurs at the same time, the homologous teeth without any treatment could allow a visual assessment and comparison to determine the discoloration of endodontically treated teeth [9]. Therefore, in this study, the homologous teeth were used as baseline. The results obtained from baseline (homologous tooth) vs up to 60 months after endodontic treatment indicate that the independent variables tested were associated with the final optical properties ($\Delta E00$ and CIEL*a*b* individual color coordinates). Thus, the hypothesis tested was accepted. The rationale for this study was the great number of *in vitro* studies with controversial results regarding the influence of endodontic treatment on crown discoloration; moreover, to the best of the authors' knowledge, this is the first clinical study reporting this issue. Findings obtained from *in vitro* conditions may not represent the actual tooth discoloration potential yielded by endodontic materials in a clinical setting [26]. The interaction of the endodontic material with salivary components and bacteria may lead to different staining mechaanisms *in vivo* [25].

To minimize the influence of subjective variables and to control methodological factors in the evaluation of $\Delta E00$, a spectrophotometric measurement was used in this study. This methodology was reported as accurate and reliable for quantitative assessments of dental color [10-12]. Variables that could interfere with the measurement of color as ambient light conditions8 [10,. 13, 16], and spectrophotometer position [10, 13, 16] were also controlled. Random errors in this study were minimized by strict control of environmental factors along with multiple measurements and mean calculation. One of the strengths of this study was the accuracy and reliability of shade records using the spectrophotometer. In addition, only one calibrated examiner performed all readings, avoiding interexaminer variability.

Different factors have been indicated as potential predictors of tooth discoloration; even so, the role played by each pointed factor remains controversial. Our results showed that the three evaluated conditions (baseline vs ≤ 1 month, baseline vs >1-12 months, and baseline vs >12 months up to 5 years) presented values of $\Delta E00 > 1.8$, which is the clinical threshold for acceptability of color.[9] The filling mate- rial, cervical limit of root filling, and tooth location playeda synergistic role in the coronal discoloration of endodontically treated teeth, with greater$\Delta E00$ found between baseline readings vs >12 months up to 5 years. Previous *in vitro* studies revealed that tooth discoloration resulting from end- odontic materials occurs from 10 days after obturation3 up to several months [2, 3, 7, 9, 12]. The difference from results could be attributed to the different methodologies, procedures, and materials used.

Regarding filling material, ZOE and MTA-based filling sealers did not differ statistically from AH Plus only in condition [1]. Nonetheless, AH Plus showed higher $\Delta E00$ values at first month after endodontic treatment than in the other periods evaluated. Findings from *in vitro* studies have reported that MTA yielded high [14, 24, 25, 27] $\Delta E00$ values in human teeth in only 4 weeks, in agreement with our results. Previous studies showed that MTA-based materials which contain bismuth oxide as radiopacifier showed severe discoloration [4, 14-16]. The ingredient bismuth trioxide seems to be responsible for this negative effect, as it reacts with collagen in den- tin matrix resulting in a grayish discoloration [4]. The coronal discoloration observed for ZOE-based endodontic materials in this study corroborates the data found

in previous *in vitro* studies [1, 13-15]. The chromogenic potential of ZOE has been associated with the relatively unstable chemical bond between zinc oxide and eugenol [14-18]. Even after the setting reaction, eugenol is released, causing self-oxidation and darkening over time [2]. AH Plus is a silver- free sealer and it has been reported that its discoloration potential is noncomparable with its predecessor AH26 [18]. A previous study showed distinct AH Plus-induced discoloration after 6 weeks, which was reduced after 8 weeks [19], and corroborates with the results of this study. Our findings showed that AH Plus originated clinically detect- able coronal discoloration in the first month of evaluation and it decreased over time. The literature available is controversial regarding the discoloration potential originated by AH Plus; while one study observed progressive unsatisfactory coronal discoloration at 6 months [20], other reported satisfactory color stability at 12 months [25].
In addition to the composition of the filling material, the cervical limit of root filling and the tooth location may also be related to coronal discoloration of endodontically treated teeth [24]. An inadequate endodontic procedure regarding the determination of the cervical limit of the endodontic material could allow the direct contact with the axial dentinal walls and the interaction between chemical components may result in darkened tooth color over time [2, 21, 24]. This optical evidence occurs mainly in anterior teeth due to their thinner dentin layer and lower masking ability [16], especially in the cervical third of the crown, because the enamel overlying this region is a translucent, colorless, and very thin structure [2]. It may explain that the higher ΔE00 observed in anterior teeth and in those with the cervical limit of root filling at the dental cervix for all conditions evaluated. However, there is a lack of scientific evidence regarding the optimal limit of the filling material in the cervical region to avoid or minimize dental chromatic alteration. Then, the complete debridement of the pulp chamber and cutting the filling material below the cemento-enamel junction, especially in the anterior aesthetic area, should be properly observed [11, 14, 16, 24]. The tooth discoloration yielded clinical aesthetic problems and discomfort for both patient and professional. Therefore, the proper choice of endodontic materials must be based on scientific evidence and the procedures on careful daily clinical practice.
In this study, measurements were performed on the cervical third of the crown, since previous studies [2, 4,-7] have been shown greater evident discoloration in this area compared to occlusal third what probably occurs due to reduced dentin thickness in this area [6]. Furthermore, other factors such as thinner gingival biotype and incidence of gingival recession may intensify the clinical appearance of cervical discoloration due to additional exposure of the dental structure to the oral environment [22].
Regarding CIEL*a*b* individual color coordinates measured, in the first month after endodontic treatment, the L*, a*, and b* values were not influenced by the difference between endodontic materials. However, the cervical limit showed lower value of L*, and anterior teeth higher value of b*, representing a tendency to yellowish coloration. After the evaluation time up to 12 months, statistical differences in the values of L* and a* were found, showing that ZOE and MTA-based sealers and cervical limit promoted lower luminosity. The a* value was influenced by the location, with the anterior teeth tending to present a reddish coloration. From the first to the second condition, it was found coronal color variation from yellow to red. In the largest time interval evaluated, from 1 to 5 years, the L* value was influenced again only by the cervical limit. However, ZOE and MTA-based filling materials and anterior teeth significantly influenced both a* and b* values, resulting in darker teeth with reddish-yellow discoloration. These results are in agreement with the report of a previous study regarding the evident dental discoloration originated from ZOE [7], in which decreased brightness was expressed in terms of decrease in value. In addition, chromatic changes from red to orange after visual evaluation of the dental crowns over time found in this study are in agreement with other studies evaluating quantitative and macroscopic clinical parameters[14] or qualitative parameters [1, 2]. Our findings are not free of limitation and should be interpreted with caution because it is the first study to investigate factors that affect the color change of endodontically treated teeth *in vivo*. The results of clinical studies are difficult to compare with those of *in vitro* studies because evaluation of the potential discoloration originated from endodontic materials in laboratory settings [2, 14-17] is based on the “worse clinical scenario” with significant amount of material in direct contact with the dentinal walls of the pulp chamber. Moreover, other factors including the type of restorative material previously used, such as amalgam, may influence tooth discoloration [7]. However, since this was a cross-sectional study, the clinical factors (eg, initial color of endodontically treated tooth) were not controlled prior to endodontic treatment. Further prospective clinical studies are required to determine long-term color variation.

Table 1: Baseline *vs* ≤1 month Baseline *vs* >1-12 months Baseline *vs* >12 months up to 5 years

Variables	*N*	Mean (*SD*)	95% CI	*P* value	*N*	Mean (*SD*)	95% CI	*P* value	*N*	Mean (*SD*)	95% CI	*P* value
Cervical limit												
Cervical	21	8.7 (6.8)	5.7-11.8	0.022*	16	8.7 (4.9)	6.1-11.3	0.037*	19	10.7 (7.0)	7.7-13.7	0.000*
2-mm	10	4.8 (1.8)	3.3-6.4		10	5.2 (1.9)	2.2-8.2		7	2.0 (0.2)	−0.8 to 4.7	
Filling material												
ZOE/MTA	19	7.6 (5.4)	5.0-10.2	0.198	14	8.6 (5.8)	5.3-12.0	0.050*	22	10.7 (6.9)	7.7-13.6	0.000*
AH Plus	12	5.7 (2.2)	3.8-7.5		12	5.0 (2.4)	2.7-7.2		4	3.2 (1.5)	−10.0 to 16.5	
Location												
Posterior	16	4.8 (2.7)	3.4-6.2	0.037*	10	4.6 (1.2)	3.8-5.5	0.005*	12	7.1 (4.0)	2.9-11.3	0.041
Anterior	15	9.6 (7.8)	5.3-14.0		16	9.9 (6.3)	6.5-13.2		14	12.4 (7.1)	8.6-16.2	

Conclusion

Higher ΔE00 values were yielded from ZOE and MTA-based filling materials, anterior teeth, and cervical limit of root filling not performed at 2 mm below the dental cervix. This indicates that end- odontic material composition and procedures should be considered to avoid postoperative tooth discoloration, mainly in anterior teeth due to their thinner structure.

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