Crown Discoloration Induced by Endodontic Sealers: Spectrophotometric Measurement of Commission International de l'Eclairage's L*, a*, b* Chromatic Parameters

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Clinical Relevance

This study emphasizes the need for attention after root canal obturation. The prevention of tooth staining post-operatively is still a clinical issue that affects patients and dentists. Clinicians must be able to recognize the etiology of local intrinsic staining and even in cases of sealer-induced discoloration must be able diagnose the type of sealer that was used. The prevention of crown discoloration in endodontically-treated teeth is currently taught in all accredited graduate and post-graduate programs.

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ABSTRACT

Despite the improvement of endodontic materials, crown discoloration induced by root

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canal sealers remains a concerning clinical issue. The aim of this study is the measurement of the alterations in CIE L*, a*, b* chromatic parameters of tooth crowns after placement of commonly used and new-generation endodontic sealers in pulp chambers. Eighty intact, fully developed third mandibular molars were randomly assigned in five groups. Crowns were cross-sectioned from the root complex 1 mm below the cement-enamel junction. The internal axial walls of the pulp chambers were debrided and coated with endodontic sealers (Roth-811, AH-26, Guttaflow, Epiphany SE). The apical access was sealed with glass-ionomer cement. The control group was only debrided. Crowns were stored in individually marked vials in standard conditions (100% humidity, 37°C). The spectral reflectance lines were recorded with a UV-Vis spectrophotometer in the visual spectrum. The CIE L*a*b* parameters were obtained by a linked computer software before sealer placement (baseline), after one week, one, three, and six months, respectively. Statistical analysis was performed with two-way mixed ANOVA models. The level of statistical significance was set at $p < 0.05$. With the exception of the control group, experimental groups presented varying chromatic alterations. Among all experimental groups, Roth-811 sealer induced the most severe alterations in CIE L*, a*, b* chromatic parameters, during all observation periods. After root canal obturation, the clinician should be aware of the presence of remaining root canal filling materials. Thorough debridement of the pulp chamber is essential for the prevention of sealer-induced discoloration.

INTRODUCTION

Tooth discoloration varies in etiology, appearance, location, severity, and affinity to tooth structure.[1] The esthetic appearance of a treated tooth still concerns the clinician and significantly affects the patient's quality of life.[2] In a clinical study, the quality of life and patient satisfaction outcomes after endodontic treatment were evaluated. Almost 10% of the patients/participants were dissatisfied with the poor esthetic appearance of the tooth involved in endodontic procedures in terms of color.[2] The effect of the esthetic appearance was equal to other effects, such as the time involved and the feeling of pain or discomfort during or after endodontic procedures.[2]

The prevention and management of coronal discoloration still remains challenging for the clinician, especially when the anterior esthetic zone is involved.

A major etiological factor for the occurrence of local intrinsic staining in the cervical and middle third of the crown is the presence of root canal filling materials in contact with the coronal dentin of the pulp chamber. Despite improvements in the physicochemical, biomechanical, and biological properties of endodontic sealers, the appearance of coronal discoloration is still evident in daily practice. Root canal obturation is the last step of root canal treatment, and coronal dentin is mainly exposed to root canal filling materials in the long term. Laboratory studies[3-7] have shown that root canal filling materials, including gutta-percha and sealers (ZOE, epoxy-based, calcium hydroxide, tricalcium phosphate), are capable of inducing mild to severe crown discoloration. Sealer-induced discoloration is time-dependent, and the severity of the chromogenic potential of the sealers depends on their chemical consistency.[4,5] To date, crown discoloration induced by silicon-based and methacrylate-based sealers has not been investigated.

Several methods have been proposed for the evaluation or measurement of endodontic-related tooth discoloration, including visual technique and color matching with a large set of color tabs (or according to the Munsell System) by experienced observers,[3-5] computer analysis of digital photos in Adobe Photoshop,[7] instrumental colorimetry,[8] and spectrophotometry.[9,10]

In daily clinical practice, the use of shade guides offers a quick and cost-effective method for measuring tooth color.[11] However, visual perception is not uniform among the observers and is considered highly subjective. Many external and physiological variables, such as the type of illumination, experience, age, fatigue of the eye, limited verbal means for the communication of visually assessed color characteristics, and metamerism, lead to inconsistencies with regard to color perception and matching.[12,13]

Several digital instruments, including dental colorimeters and spectrophotometers, have been proposed for the establishment of more precise measurements. Instrumental measurements commonly use the Commission International de I'Eclairage's (CIE's) L*, a*, b* system.[14,15] The L* values describe lightness, which ranges from black (0) to white (100), while the a* values represent red (+a*)

to green (−a*) color changes, and the b* values represent yellow (+b*) to blue (−b*) color changes.[14]

Digital colorimetry is based on the application of devices that have been set to measure tooth color with the aid of three sensors with principal sensitivities in red (R=700 nm), green (G=546 nm), and blue (B=435 nm).[16] The disadvantages of colorimeters include the design of the instrument to measure flat but not concave or convex surfaces and the significant edge-loss effects to which small aperture colorimeters are prone.[17,18] In addition, it has been shown[19-21] that interinstrument agreement is relatively poor, and colorimeters lack correlation with the visual method.

On the other hand, spectrophotometers are considered as the reference instruments in the field of color science and have been used successfully in dentistry for tooth color measurements.[22] Color measurement is based on the total reflection of crown surface in the visual spectrum. The values of spectral reflectance are used for the measurement of reflection and are graphically depicted as reflection curves. Every curve is unique for each sample.[13] Several studies[23-25] have shown that spectrophotometry is a reliable, reproducible, accurate, and quantitative method.

Currently, the alterations of CIE L*, a*, b* values due to endodontic-related causes have been measured in three laboratory studies. In two reports,[9,10] the spectrophotometric measurement of crown discoloration induced by the tetracycline-containing Ledermix paste (Lederle Laboratories, Wolfatshausen, Germany) was performed. In another study,[8] the colorimetric measurement of crown discoloration induced by the triple antibiotic paste, which is used in revascularization procedures in immature teeth, was performed. However, after a comprehensive review of the literature, no available experimental reports were found with regard to the measurement of alterations in CIE L*, a*, b* values due to sealer-induced discoloration.

The aim of this study was the spectrophotometric measurement of CIE L*, a*, b* values of tooth crowns after placement of commonly used and new-generation endodontic sealers. The null hypothesis (H_o) to be tested was that none of the sealers induce statistically significant chromatic alterations.

MATERIALS AND METHODS

Preparation of Teeth

For this study, eighty (N=80) freshly extracted, fully developed, impacted and semi-impacted third mandibular molars were collected from the Clinic of the Department of Oral and Maxillofacial Surgery, School of Dentistry, Aristotle University of Thessaloniki, Greece. All teeth were indicative of surgical or nonsurgical extraction after clinical and radiological examination. The personal details, the age, the gender, and the medical history of the donors remained confidential.

Teeth with cracks, fractures, caries, restorations, abrasions, and discoloration due to systemic intrinsic causes were excluded from the initial sample. Soft tissue was removed by soaking the teeth in 2.5% w/w NaOCl for 10 minutes. Gross calculus and stains were removed using hand scalers and pumice with polishing cups.

All teeth were cross-sectioned in the coronal third of the root complex, 1 mm below the cemento-enamel junction. The apical segments were discarded and the crowns were kept. Access cavity preparation was not performed in order to isolate the effect of the sealer as the cause of discoloration from other parameters that are not easily standardized. More precisely, the preservation of the occlusal surface aided in the prevention of alterations in optical properties due to tooth structure removal and discoloring effects by the placement of temporary and permanent filling materials or due to coronal microleakage. Other anatomical features of the pulp cavity, such as pulp horns, recessions, and pits, remained intact. Under clinical conditions, when inadequate access cavity preparation occurs those remaining areas may predispose the patient to the appearance of crown discoloration when endodontic materials remain and penetrate through incisally directed tubuli and are not accessible for thorough debridement.

Pulps were extirpated with a dental spoon, and the pulp chambers were chemo-mechanically debrided through the apical access. The internal axial walls were reamed gently with Hedstrom files No. 60-80, and irrigation was performed with 6 mL (3×2 mL) of 2.5% w/w NaOCl. The smear layer was not removed. Since ethylenediamine tetraacetic acid is used for the removal of smear layer from the root canal system, a layer of inorganic debris in the pulp chamber may exist after the removal of root canal filling materials. This may prevent or slow the possibility for diffusion of sealer chromogenic components into the tubules as a result of the occlusion of dentinal tubules. Pulp chambers were dried with sterile cotton pellets. During the collection period, all crowns were stored in distilled water at −7°C in a refrigerator.

In the beginning of the experimental period, all crowns were transferred and stored in individually marked polyethylene tubes containing distilled water (100% humidity) up to the cervix of the crown in an incubator at 37°C. Teeth were randomly assigned into four experimental groups (n=17×4) and one negative control group (n=12). The evaluated sealers were Roth-811 (Roth's International, Chicago, IL, USA) (group 1; ZnOE sealer), AH-26 (Dentsply, Maillefer, Switzerland) (group 2; epoxy resin-based sealer), Guttaflow (Roeko, Coltene, Whaledent Ltd, Germany) (group 3; polyvinylosiloxane-based sealer with incorporated gutta-percha particles), and Epiphany SE (Pentron Clinical Technologies, Wallingford, CT, USA) (group 4; methacrylate resin-based sealer).

The sealers were mixed and prepared according to the manufacturers' instructions and were placed into the pulp chambers *via* the cervical access. A finger plugger was used to coat the internal axial walls with the sealers. Mild lateral pressure was applied in order to mimic the forces applied in the pulp chamber during the lateral condensation technique. The sealer excess was removed from the apical aspect with a dry sterile cotton pellet. In group 4, light-curing was also performed in order to achieve the dual polymerization process, as it is recommended by the manufacturers. Core filling materials, including gutta-percha and Resilon cones, were not used because at the end of the obturation, the core materials are easily and predictably removed from the access cavity. On the contrary, during the application of condensation forces, sealers spread through the axial walls. The apical access was sealed with a thin layer of glass ionomer cement (Ketac Cem Aplicap; 3M, ESPE, Germany). In the control group (group 5) the pulp chambers of the crowns were filled with distilled water.

Measurement of Crown Chromatic Alterations

The chromatic alterations of all groups were measured by an ultraviolet (UV)-Vis spectrophotometer (UV-2401PC; Shimadzu Corporation, Kyoto, Japan). The UV-2401PC is a double-beam spectrophotometer, in which the light is split into two beams before it reaches the sample. Each light produced from the source lamp is passed through a monochromator, which diffracts the light into a "rainbow" of wavelengths and outputs narrow bandwidths of the diffracted spectrum. In this way, polychromatic illumination is utilized for the comparison of the light intensity between two light paths. One light path contains the beam that illuminates the sample,

and the other contains the beam that illuminates the reference sample ($BaSO_4$).

The double-beam integrating sphere was set to measure the intensity of spectral reflectance (R) of the sample assembly. Standard D_{65} illumination was chosen, as it corresponds approximately to the spectrum of midday daylight in Western/Northern Europe.[14] The spectrophotometer was linked to a computer, in which the spectral reflectance curves of the crowns, in visual spectrum (380-780 nm), were recorded. Using a specialized computer software (Color Analysis UV-2401PC), spectral curves were transformed into L* (lightness), a* (red to green), and b* (yellow to blue) values according to the CIE system.

A mounting system was developed for the customization and consistent reproducibility of the crown's position during the entire experimental period. In the black bakelite sample assembly, the cylindrical inner frame (diameter=2 cm, inner height=0.2 cm) was filled with black, nonpolychromatic, thermo-plasticized silicone. The lingual surface of each crown was fixed within the silicone mass, during the cooling phase, in order to fit in the circular opening of the aperture mask of the integrating sphere.

The dimensions of the polychromatic beam that illuminated the sample measured 7×7 mm; thus, the majority of the cervix and the crown portion was exposed and measured. The color appearance of the buccal surfaces of the crowns was measured in order to simulate their clinical appearance. During the measuring process all teeth remained wet in order to prevent enamel color alterations caused by drying.[26] Measurements were carried out by the same operator (KI).

Before the beginning of the experimental period, a pilot study[27] was conducted for the validation of the proposed method. The repeatability and precision of the mounting system were assessed, including the measurement of reproducibility and the method's error. Moreover, in the first 24 hours of the beginning of the experimental period the operator's measuring accuracy and error were recorded. The experimental model presented values of $\Delta E < 1$ unit and high positive correlation values after repeated measurements.[27] Finally, the intergroup comparison of mean (standard deviation) L_0^*, a_0^*, b_0^* values of groups 1-5 did not present statistically significant differences ($p > 0.05$); thus, the collection of the specific group of teeth was correct with regard to the initial chromatic properties of the total sample.[27]

The spectrophotometer was calibrated at each time interval, and the chromatic parameters were

measured initially, prior to the placement of the root canal sealers (baseline: t_0), and, consecutively, one week (t_1), one month (t_2), three months (t_3), and six months (t_4) after sealer placement.

All measurements were repeated twice, and mean values were recorded as soon as their difference did not exceed the estimated operator's error. Otherwise, a third measurement was taken, and the mean values that were recorded included the choice of the previous measurement that was closer to the third one, and their difference was within the levels of the operator's error.

Finally, at the end of the sixth month, two crowns from each experimental group were randomly selected and longitudinal sections were performed in the middle of their bucco-lingual aspect. Digital images of exposed dentin surface from chamber to external tooth surface of the sectioned crown specimens were taken in black font, in daylight conditions (1X1 Macro-Lense; Nikon D-80, Japan).

Statistical Analysis

Sample size was calculated after power analysis based on the results of the pilot study, according to the equation $n = 2(z_{1-\alpha/2} + z_{''\beta})^2 \sigma^2 \{1+(m-1)\rho\}/m\Delta^2 = 12$ ($\rho=0.5$, $\sigma^2=4$, $\Delta=2.2$), the chosen values of Δ and σ^2 were greater than the observed ones).[28] In the control group 12 teeth were used, and for each experimental group 17 teeth were used (additional five teeth used in case of drop out).

Two-way analysis of variance (ANOVA) with repeated measurements was used for data analysis of the values of CIE L*, a*, b* chromatic parameters. The assumptions of univariate normality were tested with Shapiro-Wilk test, and the homogeneity of variances of differences was verified with the Mauchly test of sphericity, respectively. In cases in which the hypothesis of sphericity was rejected, the Greenhouse-Geisser method was used for the computation of the *F*-statistic. The significant main effects and interactions of the experimental factors were investigated with pairwise between-group and within-group comparisons, which were conducted using the Bonferroni method. The overall analysis was performed with SPSS software (version 16.0; SPSS Inc, Chicago, IL, USA). The level of statistical significance was set at $p < 0.05$.

RESULTS

The results of the two-way ANOVA indicated that time had a significant effect on the values of L*, a*, b* chromatic parameters. The effect of the groups on the values of L*, a*, b* chromatic parameters was also statistically significant. The interactions between time and group demonstrated a significant effect with regard to the values of L*, a*, b* chromatic parameters (Table 1). The mean values of CIE L*, a*, b* parameters for all groups are presented in Table 2.

Parameter L*

Roth-811 (group 1) displayed a remarkable decrease in mean L* values at all observation periods. Within-group comparison revealed statistically significant differences between mean L_1*, L_2*, L_3*, and L_4* values compared to mean L_0* values ($p<0.05$). Mean L* decrease was more intensive one week after sealer placement. The lightness decrease was expressed as a darkening effect in the crown appearance.

AH-26 (group 2) also displayed a remarkable decrease in L* values at all observation periods. Within-group comparison revealed statistically significant differences between mean L_1*, L_2*, L_3*, and L_4* values compared to mean L_0* values ($p<0.05$). The mean L* decrease was more intensive six months after sealer placement and displayed statistically significant values in comparison to values of all previous examination times ($p<0.05$). The lightness decrease was expressed as a darkening effect in the crown appearance.

Guttaflow (group 3) did not present statistically significant changes in mean L* values during the first three months following sealer placement ($p>0.05$). After six months, a remarkable decrease was evident at a statistically significant level when compared to mean L* values of the previous examination times ($p<0.05$).

Epiphany SE (group 4) presented a statistically significant increase in L* values one week after sealer placement ($p<0.05$). The initial lightness increase was expressed as a whitening effect in the crown appearance. The levels of L* values tended to decrease and drop back to the initial L_0* values, without statistically significant differences ($p>0.05$). In the control group (group 5), mean L* values remained stable.

Intergroup comparisons of mean L* values showed that the Roth-811 group differed from the Guttaflow and Epiphany SE groups at all experimental periods ($p<0.05$). The AH-26 group differed from the Epiphany SE group after one week and one and six months into the examination period, whereas the

Source of Variation	Type III Sum of Squares	df	Mean Square	F	Significance (p Value)
Parameter L*					
Intercept	515,644.113	1	515,644.113	99,398.501	0.000
Groups	130.084	4	32.521	6.269	0.000
Error (groups)	368.323	71	5.188		
Time	212.040	3.368	62.953	59.028	0.000
Time × groups	202.223	13.473	15.010	14.074	0.000
Error (time)	255.047	239.144	1.066		
Parameter a*					
Intercept	420.557	1	420.557	264.855	0.000
Groups	94.522	4	23.631	14.882	0.000
Error (groups)	112.739	71	1.588		
Time	58.232	2.717	21.436	54.507	0.000
Time × groups	97.976	10.866	9.017	22.927	0.000
Error (time)	75.853	192.876	0.393		
Parameter b*					
Intercept	51,625.360	1	51,625.360	8072.768	0.000
Groups	63.654	4	15.914	2.488	0.051
Error (groups)	454.045	71	6.395		
Time	112.994	3.336	33.875	25.765	0.000
Time × groups	158.830	13.342	11.904	9.054	0.000
Error (time)	311.372	236.828	1.315		

Table 1: *Results of Two-way Analysis of Variance (ANOVA) for Commision Internationale d' Eclairage (CIE) L*, a*, b* Chromatic Parameters with Respect to the Effects of Groups, Time, and Their Interaction*

Table 2: *Mean (SD) values of CIE L*, a*, b* chromatic parameters.*						
Groups	**n**	**Baseline**	**1st Week**	**1st Month** **(CIE) L* parameter**	**3rd Month**	**6th Month**
		L_0^*	L_1^*	L_2^*	L_3^*	L_4^*
Roth-811(Group 1)	17	84.65 (2.17)	81.66 (3.25)	81.00 (3.64)	79.90 (3.41)	79.10 (3.32)
AH-26(Group 2)	17	83.77 (2.45)	82.75 (2.54)	82.58 (3.18)	82.45 (2.72)	80.87 (2.96)
Guttaflow(Group 3)	15	84.65 (1.61)	84.23 (1.91)	84.34 (1.80)	84.53 (2.38)	82.94 (1.73)
Epiphany SE(Group 4)	15	84.36 (1.45)	85.59 (1.44)	85.32 (1.82)	84.96 (2.25)	84.04 (2.04)
Control(Group 5)	12	82.74 (1.82)	82.58 (1.75)	82.84 (1.76)	82.30 (1.68)	81.80 (1.74)
Groups	**n**	**(CIE) a* parameter**				
		a_0^*	a_1^*	a_2^*	a_3^*	a_4^*
Roth-811(Group 1)	17	1.99 (1.00)	4.00 (0.99)	4.49 (1.18)	5.39 (1.30)	5.54 (1.15)
AH-26(Group 2)	17	1.21 (1.74)	0.74 (1.35)	0.93 (1.53)	1.26 (1.50)	1.53 (1.56)
Guttaflow(Group 3)	15	1.44 (1.27)	2.26 (1.10)	2.09 (0.96)	1.86 (1.16)	2.19 (1.00)
Epiphany SE(Group 4)	15	1.26 (1.24)	2.19 (0.93)	2.07 (1.07)	2.12 (1.39)	1.72 (1.12)
Control(Group 5)	12	2.36 (1.91)	2.47 (1.91)	2.62 (1.75)	2.59 (1.65)	2.96 (1.47)
Groups	**n**	**(CIE) b* parameter**				
		b_0^*	b_1^*	b_2^*	b_3^*	b_4^*
Roth-811(Group 1)	17	24.89 (2.18)	27.53 (2.98)	27.73 (2.53)	28.94 (1.96)	25.77 (2.02)
AH-26(Group 2)	17	24.75 (2.14)	24.20 (1.93)	25.18 (2.31)	25.17 (2.45)	25.50 (2.17)
Guttaflow(Group 3)	15	25.83 (2.73)	26.69 (2.83)	26.85 (1.98)	27.04 (2.30)	26.20 (2.24)
Epiphany SE(Group 4)	15	26.10 (2.41)	28.24 (2.14)	28.60 (2.03)	27.41 (1.83)	26.59 (2.32)
Control(Group 5)	12	25.05 (4.17)	25.66 (4.30)	25.46 (4.36)	25.40 (4.56)	26.07 (3.95)

Epiphany Se group differed from the control group after one week of examination.

Parameter a*

Roth-811 (group 1) displayed a remarkable increase in mean a* values at all observation periods. This finding is expressed as an increase in redness effect in the crown appearance. Within-group comparison revealed statistically significant differences between mean a_1^*, a_2^*, a_3^*, and a_4^* values compared to mean a_0^* values ($p < 0.05$). Mean a* increase was more intense one week after sealer placement.

AH-26 (group 2) did not induce significant alterations in mean a* values. Guttaflow (group 3) presented a significant increase in mean a* values up to the first month of observation, in comparison to mean a_0* ($p<0.05$).

Epiphany SE (group 4) presented a significant increase in mean a* values up to the third month of observation, in comparison to mean a_0* ($p<0.05$). In the control group (group 5) mean a* values remained stable.

Intergroup comparisons of mean a* values showed that the Roth-811 group differed from the AH-26, Guttaflow, Epiphany SE, and control groups at all experimental periods ($p<0.05$). After one week, the AH-26 group differed from the Guttaflow and Epiphany SE ($p<0.05$) groups, and, additionally, it differed from the control group after one week and one and six months into the examination ($p<0.05$).

Parameter b*

Roth-811 (group 1) displayed a remarkable increase in b* values after three months of examination. This finding is expressed as an increase in yellowness of the crown appearance. Within-group comparison revealed statistically significant differences between mean b_1*, b_2*, and b_3* values compared to mean b_0* value ($p<0.05$). The mean b* increase was more intense one week after sealer placement.

AH-26 (group 2) displayed a stable b* value. A significant increase was observed in mean b* values from the first week to the first month of observation, consecutively ($p<0.05$).

Guttaflow (group 3) presented a significant increase in mean b* values in the first and third months of examination in comparison to mean b_0* ($p<0.05$).

Epiphany SE (group 4) presented a significant increase in mean b* values after one month of examination ($p<0.05$). Within-group comparison revealed statistically significant differences between mean b_1* and b_2* values compared to mean b_0* value ($p<0.05$). In the control group (group 5), mean b* values remained stable.

Intergroup comparisons of mean b* values showed that the Roth-811 group differed from the AH-26 group after one week and three months of examination and from the control group after three months of examination ($p<0.05$). The AH-26 group differed from the Epiphany SE group after one week and one month of examination ($p<0.05$), whereas the Epiph-

any SE group differed from the control group after one month of examination ($p<0.05$).

DISCUSSION

One of the major advantages of visual spectrophotometry is that tooth color measurement is based on the measurement of spectral reflectance in visual spectrum.[15] All of the methods that have been proposed in previous studies, which either require visual shade assessment or perception or which lack standardization, may be improved by the use of a spectrophotometer, which would allow these methods to overcome their inherent disadvantages. However, a major requirement for the application of instrumental measurements is the minimization of procedural errors and the control of methodological factors in order to reduce the amount of uncertainty during the measuring process.

The collection of freshly extracted, fully developed impacted and semi-impacted mandibular molars was selected because these teeth are unaffected by intraoral exogenous factors that may pose an effect on their optical properties. In addition, the availability of these teeth is prominent. Although these teeth are not representative of the anterior esthetic zone, the choice of the specific sample was correct, since the initial chromatic and optical properties among groups 1-5 did not differ. On the contrary, in all previous studies,[3-7] freshly extracted, non-decayed, single-rooted premolars or anterior maxillary teeth were used, and it was taken for granted that the initial chromatic properties of the selected teeth were equal, regardless of the crown size and the dental tissue thickness.

The sole measurement of CIE color differences from the equation $\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$ allows for a mathematical comparison but does not indicate the direction of the color differences. This is the first study in which the effects of the remaining sealers on crown color were measured according to the CIE L*, a*, b* system. The comparison of the CIE chromatic parameters allows for the quantification of color differences that can be expressed in terms of human visual perception.

As was shown in a preliminary study,[27] Roth-811 sealer induced perceptible crown discoloration after one week of examination. The descriptive analysis of the results of this study showed intense color alteration, including a decrease in lightness and an increase in the intensity of redness and yellowness. The visual assessment of tooth crowns in group 1 revealed that teeth became darker; this was accom-
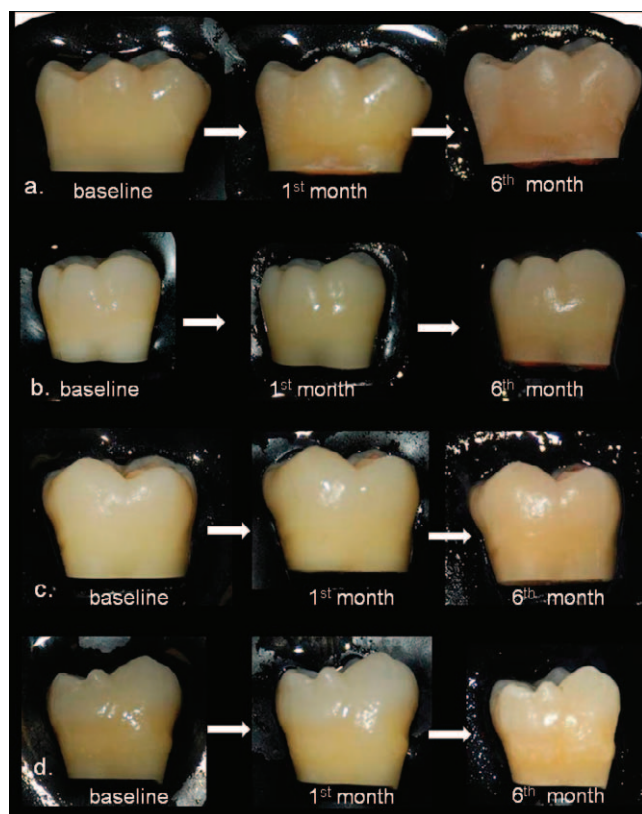
Figure 1. *Buccal views of crown specimens*
   (a) Group 1 (Roth-811). Severe lightness decrease and yellowish-reddish chromatic alteration extending from root cervix to middle third of crown specimen.
   (b) Group 2 (AH-26). Moderate lightness decrease in root cervix and cervical third of crown specimen.
   (c) Group 3 (Guttaflow). Minor increase in yellowness-redness of crown specimen.
   (d) Group 4 (Epiphany SE). Minor initial lightness increase (1st month) and rebound of tooth color (6th month) of crown specimen.

panied by yellow-reddish discoloration initially at the crown cervix and laterally extending at the middle third of the crown (Figure 1a). The darkening effect of ZOE sealers was evident in a laboratory study[6] in which the lightness decrease was expressed in terms of value decrease, according to the Munsell system. Moreover, both the quantitative and macroscopical clinical findings of this study are in agreement with the qualitative findings of previous studies[4,5] that reported red to orange chromatic alterations after visual assessment of tooth crowns.

The chromogenic potential of ZOE sealers has been attributed to the unstable chemical bond between ZnO and eugenol.[29] Even after the end of the setting reaction, eugenol release leads to self-oxidation and becomes darker with time.[30] Moreover, ZOE sealers present increased solubility.[31,32]

This type of crown discoloration has been characterized as chemical discoloration.[29] Clinicians should be aware of the presence of ZOE sealer remnants, especially in the anterior esthetic zone. Thorough debridement of the pulp chamber is essential to prevent or reduce the possibility of discoloration rise.

AH-26 sealer induced darkening of the crowns, which was quantitatively expressed as a lightness decrease during all examination periods. Our findings are in agreement with the results of previous studies.[4-6] On the contrary, alterations in CIE a* and b* chromatic parameters were negligible during all examination periods. A characteristic qualitative macroscopic finding was the alteration of the sealer's color from deep yellow to black after setting, which affected the cervical third of the crowns (Figure 1b). This is in accordance with previous laboratory findings,[6] in which sealer structure and color were assessed after longitudinal sections of tooth crowns filled with sealers.

In the first generation of silver-containing AH-26 sealer, the chromogenic potential was attributed to the release of silver ions during and following the setting reaction.[4] Although manufacturers claim that new-generation epoxy-based sealers do not contain silver ions, presumably other ingredients or substances that affect the polymerization process and that have not been evaluated may play a role in the mechanism of sealer color alteration and tooth discoloration.

The results of a preliminary study[27] showed that Guttaflow and Epiphany SE sealers did not induce perceptible chromatic alterations. The descriptive analysis of the results of this study showed that slight tooth darkening was observed in group 3 only after six months of examination (Figure 1c). However, the severity of the darkening effect of Guttaflow was insignificant in comparison to the severity of the darkening effects of Roth-811 and AH-26 sealers. One concern for the examination of Guttaflow was related to the chromogenic potential of the incorporated particles of gutta-percha. The visual assessment of crowns by experienced observers showed that gutta-percha induced mild pinkish discoloration after 15 weeks of observation.[3] A slight tendency to redness and yellowness was initially observed during the first month of examination; however, it did not pose a direct effect in the color of the crowns in group 3.

Interestingly, Epiphany SE was the only sealer that induced an initial lightness increase in the crowns of group 4 during the first month of
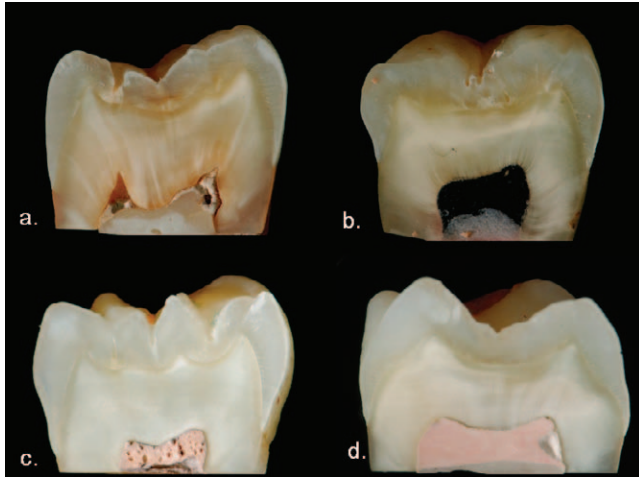
Figure 2.   *Longitudinal sections of crown specimens after six months*
   (a) Group 1 (Roth-811). Granular, grayish appearance of sealer's mass. Dark orange dentinal staining and varying leaching within tubules in areas where sealer remnants were in contact with the axial walls of the pulp chamber.
   (b) Group 2 (AH-26). Granular black appearance of sealer's mass. Grayish to black dentinal staining evident in areas where sealer remnants were in contact with the axial walls of the pulp chamber.
   (c) Group 3 (Guttaflow), (d) Group 4 (Epiphany SE). Absence of dentinal staining and sealers' color stability.

examination; thus, the descriptive analysis of the results showed that teeth became whiter in the short term. After six months, the levels of L* values displayed a rebound back to their baseline values (Figure 1d). On a theoretical basis, it can be assumed that the addition or the incorporation of nonspecified chromogenic chemical agents may contribute to the improvement of the optical properties of dentin. Further investigation of the physicochemical and optical properties of the sealer's resinous matrix is requisite.

The overall mechanism of crown discoloration due to the presence of remaining endodontic materials still remains contradictory. Kraus and Jordan[33] first stated that the pathway by which staining materials diffuse from the canal is through dentinal tubules, which are incisally directed in the tooth cervix. The increased translucency of enamel and the relative reduction of dentine thickness in that area would explain why discoloration is more evident in the cervical area.[34,35] Moreover, other factors, such as the thin gum biotype and the incidence of gum recession, may intensify the clinical appearance of cervical discoloration due to additional exposure of tooth structure to the oral environment.[33] On the other hand, the *in vitro* study of Davis and others[6] refuted the theory of sealer leaching into dentinal tubules over time. Although tooth staining was evident, sealer chromogenic substances were only

visible in the predentine (0-100 μm) in the presence of smear layer. It should be noted that sealers were placed in a passive way within the pulp chambers without further application of lateral forces.

In this study, the visual evaluation of the longitudinal sections (1×1) in the experimental groups displayed varying results, which depended on the type of root canal sealer. More specifically, in group 1 (Roth-811) the set sealer displayed a granular, grayish appearance. A dark orange dentinal staining accompanied by varying leaching within tubules was evident in areas where sealer remnants were in contact with the axial walls of the pulp chamber (Figure 2a). In group 2 (AH-26) the set sealer displayed a granular black appearance. A grayish to black dentinal staining was also evident (Figure 2b). Apart from the induction of chemical discoloration of dentine, an additional explanation for the findings in groups 1 and 2 is the application of mild lateral forces in the axial walls during the sealer placement procedure in all experimental groups.

On the contrary, dentinal staining was not evident either in group 3 (Guttaflow) or in group 4 (Epiphany SE). After six months of examination, the incorporated gutta-percha and the polyvinylosiloxane matrix of set Guttaflow and the methacrylate resinous matrix of set Epiphany SE did not display any chromatic alterations in comparison to their color appearance during the mixing and placing procedures (Figure 2c,d). This finding confirms the acceptable and enhanced physicochemical properties of those two new-generation sealers with regard to their chemical stability.[32,36] The slight alterations of CIE L*, a*, b* values of the crowns may be attributed to the alterations in the optical properties of the internal dentinal surface due to direct sealer mechanical exposure and reduction of dentine translucency. Guttaflow and Epiphany SE sealers posed the least risk for potential discoloration effects.

Ideally, in order not to impart discoloration, the set sealer should have increased lightness, yellow to yellow-red chromatic appearance similar to the color of dentin, and a similar translucency parameter, mimicking the optical properties of dentin.[6]

CONCLUSIONS

Roth-811 and AH-26 sealers promoted darkening effects in crowns. On the contrary, Guttaflow and Epiphany SE sealers posed the least risk for potential discoloration effects. Apart from basic

properties such as biocompatibility and good sealing ability, this study indicates that the chromogenic potential of endodontic sealers may also play an important role in selecting proper root canal filling materials.

Conflict of Interest

The authors deny any conflicts of financial interest. This study is part of a MSc. Thesis.

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