Clin Oral Invest (2013) 17:1525–1533
DOI 10.1007/s00784-012-0850-x

ORIGINAL ARTICLE

# Validation and spectrophotometric analysis of crown discoloration induced by root canal sealers

**Konstantinos Ioannidis · Panagiotis Beltes · Theodor Lambrianidis · Dimitrios Kapagiannidis · Vassilis Karagiannis**

Received: 16 January 2012 / Accepted: 19 September 2012 / Published online: 30 September 2012


**Abstract**
*Objectives* A major cause of crown discoloration is root canal sealer remnants in the pulp chamber, after root canal obturation. The aim of this study was the evaluation of the chromogenic effect of common and new-generation root canal sealers. The tested null hypothesis was that none of the sealers induced clinically perceptible crown discoloration, ex vivo ($H_0$: CIE color difference $\Delta E<3.7$).
*Methods* The crowns of 80 intact, fully developed third mandibular molars were cross-sectioned 1 mm below the cementoenamel junction and stored in standard conditions (100 % humidity, 37 °C). The pulp chambers were chemomechanically debrided with hand files and sodium hypochlorite (NaOCl) and the specimens were randomly assigned into four experimental (1–4) and one control (5) groups. In the experimental groups, the internal axial walls of the pulp chambers were coated with sealers (Group 1; Roth 811, Group 2; AH26, Group 3; GuttaFlow, Group 4; Epiphany SE). The pulp chambers of the control group (Group 5) remained unfilled. The spectral reflectance lines of the crowns were recorded by a UV–Vis spectrophotometer. CIE total color differences $(E = [(L*)^2 + (a*)^2 + (b*)^2]^{\frac{1}{2}})$ were calculated after 1 week, 1 month, 3 months, and 6 months, respectively. A pilot study was also conducted for the validation of the experimental model. Two-way mixed ANOVA models were used for statistical data analysis.
*Results* The experimental model presented values of $\Delta E<1$ unit and high positive correlation values, after repeated measurements ($p<0.05$). Roth 811 sealer (Group 1) induced clinically perceptible crown discoloration ($\Delta E>3.7$, $p<0.05$) and presented statistically significant differences from Groups 2–5 in all examination intervals ($p<0.05$). In Groups 2–5, clinically perceptible crown discoloration was not observed ($\Delta E<3.7$, $p<0.05$).
*Conclusions* Roth 811 sealer exhibited severe discoloration effects. Within the limitations of this study, it can be concluded that any sealer remnants should be removed from the pulp chamber after root canal obturation and before placement of a final restoration.
*Clinical relevance* The chromogenic potential may play an important role in selecting proper root canal sealers.

**Keywords** Chromogenic potential · CIE color system · Crown discoloration · Root canal sealer · Spectrophotometry

K. Ioannidis (✉)
300 Vauxhall Bridge Road SW1V 1AA,
London, UK
e-mail: pabloioannidis@yahoo.com

P. Beltes · T. Lambrianidis
Department of Endodontology, School of Dentistry, Aristotle University of Thessaloniki,
Thessaloniki, Greece

D. Kapagiannidis
Thessaloniki, Greece

V. Karagiannis
Department of Mathematics, Aristotle University of Thessaloniki,
Thessaloniki, Greece

## Introduction

Poor aesthetic appearance of teeth involved in endodontic procedures is an ongoing concern for clinicians that significantly affects patients' quality of life [1]. Occurrence of local intrinsic crown discoloration may exist prior to root canal treatment due to pulp bleeding and subsequent necrosis, dystrophic calcification after dental trauma, or aging [2, 3]. After root canal treatment, discoloration may arise as a

result of the interaction of root canal irrigants or after placement of antibiotic pastes, repair, and temporary and root canal filling materials [2, 3].

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 [3]. On the long-term, core materials and sealers interact with dentin. Any change to the optical and chromatic properties of the dentinal structure is likely to cause an alteration in the outward appearance of the crown caused by its light transmitting and reflecting properties [2].

The reduction of the coronal aspect of the root canal filling below the clinical cervix is essential for the prevention of sealer-induced crown discoloration in the anterior aesthetic zone. However, sealer remnants cannot be always thoroughly removed from the pulp chamber in case of limited visualization when an operating microscope is not used. Sometimes they are present due to clinician's negligence to provide adequate in-depth mechanical and chemical cleaning of the cervical and coronal axial dentinal walls after root canal obturation. Several laboratory studies have shown that sealer remnants (ZnOE, epoxy-based, calcium hydroxide, tricalcium phosphate) are capable of inducing mild, moderate, or severe crown discoloration [4–8]. Sealer-induced discoloration is progressive and the severity is associated with their chemical consistency [5, 6]. The chromogenic potential of new-generation sealers, including silicon-based and methacrylate-based sealers, has not been investigated yet.

Several methods have been proposed for evaluation or measurement of sealer-induced crown discoloration, including visual assessment and color matching with sets of color tabs in standard light conditions [4–6], evaluation of dentin color alterations in longitudinal sections of pulp chambers [7], and computer analysis of digital photos [8]. Digital colorimeters and spectrophotometers are also available for objective color measurement in clinical and laboratory conditions [9]. Spectrophotometers are considered as reference instruments in the field of color science and have been used successfully in dentistry for quantitative tooth color measurements [10–13]. Spectrophotometry has not been used for investigation of sealer-induced discoloration yet.

Instrumental measurements, including dental colorimeters and spectrophotometers, commonly utilize the Commission International de I'Eclairage's (CIE) $L^*a^*b^*$ system [14]. CIE is an organization recognized by ISO as an international standardization body on the subject of light, vision, and color. The CIE $L^*a^*b^*$ system is a three-dimensional, uniform colored space with $L^*$ (lightness), $a^*$ (red to green), and $b^*$ (yellow to blue) parameters. The total color difference between two objects is expressed numerically by their Euclidean distance, in $\Delta E$ values [14].

The aims of this study are: (1) the performance of a pilot study for the validation of a novel experimental method and (2) the investigation of chromatic alterations in tooth crowns induced by commonly used and new-generation sealers, including spectrophotometric measurement of $\Delta E$ units, in the CIE color system. The research hypothesis to be tested was that none of the tested sealers induced perceptible chromatic alterations.

## Materials and methods

### Preparation of teeth

For this study, 80 ($N$=80) intact freshly extracted, fully developed, impacted, and semi-impacted third mandibular molars were collected from the Clinic of Oral and Maxillofacial Surgery (School of Dentistry, Aristotle University of Thessaloniki, Greece). The teeth were indicative of extraction after clinical and radiological examinations. Personal details, age, gender, and medical history of the donors remained confidential. All procedures were contacted in accordance with the protocol outlined by the Ethical Committee of the School of Dentistry and the Research Committee, Aristotle University of Thessaloniki, Greece.

Soft tissue was removed by using hand scalers after soaking the teeth in 2.5 % sodium hypochlorite (NaOCl) for 10 min. Access cavity preparation was not performed. Teeth were cross-sectioned in the coronal third of the root complex, 1 mm below the cementoenamel junction. The apical segments were discarded and the crowns were preserved. The internal axial walls of the pulp chambers were chemomechanically debrided with Hedstrom files (No. #60-80) and 2.5 % w/w NaOCl (6 mL) through the apical access. No attempt was performed to remove the smear layer.

Crowns were stored in individually marked polyethylene tubes containing distilled water (100 % humidity) up to the cervix of the crown in an incubator at 37 °C. The specimens were randomly assigned to four experimental and one negative control groups. The evaluated sealers were: Roth 811 (Roth International, Chicago, IL, USA) (Group 1; ZnOE sealer / $n_1$=17), AH-26 (Dentsply, Maillefer, Ballaigues, Switzerland) (Group 2; epoxy resin-based sealer / $n_2$=17), GuttaFlow (Roeko, Coltene, Whaledent Ltd., Langenau, Germany) (Group 3; polyvinilosiloxane-based sealer with incorporated gutta-percha particles / $n_3$=17), Epiphany SE (Pentron Clinical Technologies, Wallingford, CT, USA) (Group 4; methacrylate resin-based sealer / $n_4$=17).

The sealers were prepared according to the manufacturer's instructions. A finger plugger was used to coat the internal axial walls with the sealers via the cervical access. A mild lateral pressure was applied in order to mimic the applied forces in the pulp chamber during the lateral

condensation technique. In Group 4, light curing was also performed in order to achieve the dual polymerization process. The apical access was sealed with a thin layer of glass ionomer cement (Ketac Cem Aplicap, 3M, ESPE, Seefeld, Germany). The pulp chambers of the control group (Group 5 / $n_5$=12) were filled with distilled water.

### Measurement of chromatic alterations

The chromatic alterations were measured by a double-beam UV–Vis spectrophotometer (UV-2401PC, Shimadzu Corporation, Kyoto, Japan). Standard $D_{65}$ illumination (Western/Northern Europe midday daylight) was chosen [14]. The spectrophotometer was linked to a computer, which recorded the spectral reflectance curves of the crowns, in visual spectrum (380–780 nm). In a specific software (Color Analysis UV-2401PC, Shimadzu Corporation, Kyoto, Japan), spectral curves were transformed into CIE $L^*$, $a^*$, and $b^*$ values.

The spectrophotometer was calibrated at each time interval and measurements were performed prior to the placement of the root canal sealers (baseline: $t_0$) and consecutively 1 week ($t_1$), 1 month ($t_2$), 3 months ($t_3$), and 6 months ($t_4$) after sealer placement. Total color differences ($\Delta E$) were measured according to the equation: $E_i = \left[(L_i - L_0*)^2 + (a_i - a_0*)^2 + (b_i - b_0*)^2\right]^{\frac{1}{2}}$($_i$ = values of parameters at $t_1$, $t_2$, $t_3$, $t_{4/0}$=values of parameters at $t_0$). The proposed acceptance for color matching adopted in this study was at 3.7$\Delta E$ units (perceptibility threshold), beyond which the differences are clinically perceivable [15].

A mounting system was developed for the customization and the reproducibility of each crown's position. In the black bakelite sample assembly, the cylindrical inner frame (diameter=2 cm, inner height=0.2 cm) was filled with black, thermoplasticized 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 dimension of the polychromatic beam that illuminated the sample was 7× 7 mm. The majority of the buccal surface of the crown and the cervix was exposed and measured in order to simulate clinical appearance. During measurements, crowns were immediately (<1 min) transferred from their polyethylene tubes to the spectrophotometer in order to retain their humidity and prevent procedural enamel color alterations caused by drying [16].

A pilot study was conducted for the validation of the proposed method, before the experimental measurements. Ten specimens ($n$=10) were randomly collected from the initial sample. The effect of the mounting system on the alterations of CIE $L^*$, $a^*$, and $b^*$ parameters and $\Delta E$ differences was evaluated in three observation periods (A: initially, B: after 48 h, and C: after 1 week). In the beginning of the experimental period, the operator's measuring accuracy and error were recorded initially (baseline: $t_0$) and after 24 h ($t_{24\ h}$). After the pilot study, the teeth were rearranged in the sample and further randomization was performed in order to create the four experimental and the one control groups.

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 performed.

### Statistical analysis

In the pilot study, reproducibility and operator's measuring accuracy were measured with Pearson's $r$ correlation coefficient. The agreement between the measurements was estimated by limits of disagreement [17]. Dahlberg's formula was used to estimate the precision of the method and the operator's error. One sample $t$ test was used to test the null hypothesis that mean $\Delta E$ chromatic differences were equal to the perceptibility threshold ($H_0$: $\Delta E_{AB,\ BC,\ AC}$=3.7). Mean $L_0^*$, $a_0^*$, and $b_0^*$ values of all groups were subjected to intergroup comparisons for the investigation of the equality of the initial optical properties of the included teeth.

Sample size was calculated after power analysis based on the results of the pilot study, according to the equation: $n = 2\tilde{}(z_{1-\alpha/2} + z_\beta)^2\sigma^2\{1 + (m-1)\rho\}/m^2 = 12$ ($\rho$=0.5, $\sigma^2$=4, $\Delta$=2.2, the chosen values of $\Delta$ and $\sigma^2$ were greater than the observed ones) [18]. In the control group, 12 teeth were used. For each experimental group, 17 teeth were used to enhance the reliability of the results, since measurements were performed prospectively.

Two-way ANOVA with repeated measurements was used for data analysis of the color differences ($\Delta E$). The significant effects and interactions of the experimental factors were investigated with pairwise comparisons, which were conducted with Bonferroni's 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. The null hypothesis to be tested was that none of the tested sealers induced crown discoloration that exceeded the perceptibility threshold ($H_0$: $\Delta E$<3.7, $p$<0.05).

## Results

### Pilot study

The results of the pilot study are presented in Table 1. With regard to the CIE $L^*$, $a^*$, and $b^*$ values, the method's error (accuracy) ranged from 0.17 to 0.79 U (<1 U) for all

**Table 1** Results of the pilot study. **a** Accuracy of the mounting system for the customization of the crown's position. **b** Comparison of mean total color differences ($\Delta E$) (one sample *t* test). **c** Measurement of accuracy and operator's error. **d** Intergroup comparison of initial mean (SD) CIE color parameters ($L_0$*, $a_0$*, $b_0$*)

| 1a | Observation period | Mean (SD) | Limits of disagreement | Dahlberg's formula | Pearson's correlation coefficient | | |
|---|---|---|---|---|---|---|---|
| $\Delta L$* | A vs. B | 0.10 (0.44) | −0.78, 0.98 | 0.30 | 0.98 | | |
| | B vs. C | 0.03 (0.26) | −0.55, 0.48 | 0.17 | 0.99 | | |
| | A vs. C | 0.07 (0.35) | −0.64, 0.77 | 0.24 | 0.99 | | |
| $\Delta a$* | A vs. B | 0.14 (0.25) | −0.64, 0.36 | 0.19 | 0.97 | | |
| | B vs. C | 0.46 (0.26) | −0.07, 0.99 | 0.37 | 0.97 | | |
| | A vs. C | 0.32 (0.20) | −0.08, 0.72 | 0.26 | 0.99 | | |
| $\Delta b$* | A vs. B | 0.40 (0.70) | −0.98, 1.78 | 0.54 | 0.96 | | |
| | B vs. C | 0.50 (1.00) | −2.62, 1.53 | 0.79 | 0.87 | | |
| | A vs. C | 0.14 (0.80) | −1.74, 1.46 | 0.55 | 0.95 | | |
| 1b | Mean (SD) | *p* values | | | | | |
| $\Delta E_{AB}$ | 0.77 (0.54) | <0.001 | | | | | |
| $\Delta E_{BC}$ | 0.83 (0.42) | <0.001 | | | | | |
| $\Delta E_{AC}$ | 1.10 (0.68) | <0.001 | | | | | |
| 1c | Mean (SD) | Dahlberg's formula | Pearson's correlation coefficient | *p* values | | | |
| $\Delta L$* ($t_0$ vs. $t_{24\ h}$) | 0.23 (0.76) | 0.56 | 0.97 | $p<0.001$ | | | |
| $\Delta a$* ($t_0$ vs. $t_{24\ h}$) | 0.23 (0.47) | 0.37 | 0.97 | $p<0.001$ | | | |
| $\Delta b$* ($t_0$ vs. $t_{24\ h}$) | 0.50 (0.64) | 0.57 | 0.98 | $p<0.001$ | | | |
| 1d | Groups | Roth 811 | AH26 | GuttaFlow | Epiphany SE | Control | *p* values |
| CIE parameters | | | | | | | |
| $L_0$* | | 84.60 (2.17) | 83.77 (2.45) | 84.65 (1.60) | 84.36 (1.45) | 82.79 (1.82) | $p>0.05$ |
| $a_0$* | | 1.98 (1.00) | 1.21 (1.74) | 1.44 (1.27) | 1.26 (1.24) | 2.36 (1.9) | $p>0.05$ |
| $b_0$* | | 24.90 (2.18) | 24.75 (2.14) | 25.83 (2.73) | 26.10 (2.41) | 25.05 (4.16) | $p>0.05$ |

observation periods. The values were included within the limits of disagreement (Table 1a). The evaluation of the reproducibility of the experimental method showed high positive correlation values for all chromatic parameters (Table 1a, Pearson's Correlation Coefficient=0.87–0.99). Mean (SD) $\Delta E_{AB,\ BC,\ AC}$ values were statistically significant from the perceptibility threshold ($\Delta E$=3.7, $p<0.05$) (Table 1b). Operator's error for CIE $L^*$, $a^*$, and $b^*$ values was found to range from 0.37 to 0.57 U (<1 U). Operator's reproducibility showed high positive correlation values (Table 1c, Pearson's Correlation Coefficient=0.97–0.99). The intergroup comparison of mean (SD) $L_0^*$, $a_0^*$, and $b_0^*$ values 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 (Table 1d).

### Experimental study

The results of the two-way ANOVA for the total color differences ($\Delta E$) with respect to the effects of time, groups, and their interaction are presented in Table 2. Time had a significant effect on the values of total color differences ($\Delta E$) [$F(2.245, 159.417)=18.827$, $p<0.001$]. The effect of the groups to the values of total color differences ($\Delta E$) was also statistically significant [$F(4, 71)=61.969$, $p<0.001$]. The interactions between time and groups demonstrated a significant effect to the values of total color differences ($\Delta E$) [$F(8.981, 159.417)=8.603$, $p<0.001$].

The results of mean (SD) total color differences ($\Delta E$) of all groups are presented in Table 3. Two crowns were excluded from Groups 3 and 4 due to enamel–dentin fracture and loss of customization from the mounting system. One week after sealer placement, mean $\Delta E$ of Roth 811 (Group 1) exceeded the perceptibility threshold ($p<0.05$). Roth 811 (Group 1) displayed the most severe discoloration in comparison with Groups 2–5. Intergroup comparisons showed that mean $\Delta E$ values of Roth 811 were statistically different from mean $\Delta E$ values of Groups 2–5 at all observation periods ($p<0.05$).

Experimental Groups 2–4 did not exceed the perceptibility threshold at any examination period. After 6 months, mean $\Delta E_4$ value of AH-26 (Group 2) was close to the perceptibility threshold, without exceeding it. However, after within-group comparisons, statistically significant values were observed with $\Delta E$ values of the previous examination periods ($p<0.05$).

Mean $\Delta E$ values of the control group (Group 5) reached 1 U during the first 3 months. After 6 months, mean $\Delta E_4$ was statistically significant in comparison with mean $\Delta E_1$ values ($p<0.05$).

## Discussion

The application of spectrophotometry in several laboratory and clinical studies offers the opportunity for quantitative measurements and data comparison as soon as accuracy and reproducibility are accomplished [11–13]. A major requirement for the application of instrumental measurements is the minimization of systematic and procedural errors. Systematic errors cannot be totally controlled, are inherent in all instruments, and may result from fluorescence, instrument metamerism, and variations in measurement geometry [19]. On the contrary, procedural and random errors can be minimized by controlling several methodological and environmental factors in order to reduce the amount of uncertainty during the measurement. The pilot study confirmed the validity of this novel laboratory methodology. However, the differences in the previous experimental protocols for the reproduction of crown discoloration and the lack of methodological standardization for the precise evaluation or measurement of color alterations still exist and have not been overcome yet; thus, the available scientific data from previous studies present an inherent heterogeneity.

In this laboratory study, the investigation of the discoloration potential of root canal sealers was based on the generation of a "worst case scenario" by leaving a significant amount of sealers in direct contact with the axial dentinal walls and several anatomical features of the pulp chamber.

**Table 2** Results of two-way ANOVA for color differences ($\Delta E$) with respect to the effects of groups, time, and their interaction

| Source of variation | Type III sum of squares | df | Mean square | $F$ | Significance ($p$ value) |
|---|---|---|---|---|---|
| Color differences ($\Delta E$) | | | | | |
| Intercept | 703.867 | 1 | 703.867 | 796.872 | 0.000 |
| Groups | 218.946 | 4 | 54.736 | 61.969 | 0.000 |
| Error (groups) | 62.713 | 71 | 0.883 | | |
| Time | 42.273 | 2.245 | 18.827 | 15.555 | 0.000 |
| Time×Groups (interaction) | 77.265 | 8.981 | 8.603 | 7.108 | 0.000 |
| Error (time) | 192.951 | 159.417 | 1.210 | | |

**Table 3** Mean (SD) $\Delta E$ values of Groups 1–5 in all examination periods

| Groups | Number | 1st week ($\Delta E_{1,\ t1-t0}$) | 1st month ($\Delta E_{2,\ t2-t0}$) | 3rd month ($\Delta E_{3,\ t3-t0}$) | 6th month ($\Delta E_{4,\ t4-t0}$) |
|---|---|---|---|---|---|
| Roth 811 (Group 1) | 17 | 4.95 (1.65)[a] | 5.60 (1.68)[a] | 7.52 (1.92)[a] | 6.88 (1.88)[a] |
| AH-26 (Group 2) | 17 | 2.07 (0.86) | 2.34 (0.99) | 2.42 (0.06) | 3.54 (1.24)[b] |
| GuttaFlow (Group 3) | 15 | 2.03 (0.82) | 2.19 (0.89) | 2.63 (1.33) | 3.04 (1.70) |
| Epiphany SE (Group 4) | 15 | 3.07 (0.94) | 3.10 (0.91) | 2.83 (1.17) | 2.11 (1.10) |
| Control (Group 5) | 12 | 0.77 (0.45) | 1.03 (0.54) | 1.11 (0.63) | 2.12 (1.12)[c] |

[a] Statistically significant differences after intergroup comparisons between Group 1 and Groups 2–5

[b] Statistically significant differences after within-group comparisons (Group 2) between $\Delta E_4$(G2) and $\Delta E_1$(G2), $\Delta E_2$(G2), $\Delta E_3$(G2)

[c] Statistically significant differences after within-group comparisons (Group 5) between $\Delta E_4$(G5) and $\Delta E_1$(G5)

The results of this study do not directly represent the in vivo tooth discoloration potential of root canal sealers in good clinical practice. However, the knowledge of the magnitude of the sealers' chromogenic potential indicates that thorough cleaning measures should be considered to prevent discoloration postoperatively.

Different tooth groups present different responses to dental materials in terms of induced discoloration due to variations in tooth morphology; size; shape; arrangement of enamel prisms, enamel, and dentine layer thickness; and dentine tubules thickness [20, 21]. Although the concerns of the stated clinical problem affect the anterior esthetic zone, the collection of 80 freshly extracted maxillary incisors was an unrealistic target, considering the scarcity of these teeth in an intact morphological, anatomical, and color condition to use in a laboratory study. The collection of freshly extracted, fully developed, impacted, and semi-impacted mandibular third molars was decided as a result because the availability of these teeth is abundant. The teeth were immediately collected from the surgical operational field and they were unaffected by intraoral exogenous factors such as aging, decay, extrinsic stains, calculus, and abrasions that may pose an effect on their optical properties. Among Groups 1–5, the chromatic parameters $L_0$*, $a_0$*, and $b_0$* did not differ at levels of statistical significance, while they were homogeneous regarding the sample's homogeneity tests (Shapiro–Wilk). Thus, the assessment of the initial sample's chromatic properties, size, and shape homogeneity revealed that the specific sample included appropriate subjects for this study.

Following root sectioning, access cavity preparation was not performed in order to isolate the discoloring effects of the root canal sealers within the pulp chambers. Several anatomical features of the pulp cavity such as pulp horns, recessions, and pits remained intact. In clinical practice, if these areas remain after inadequate access cavity preparation, then they are not accessible for thorough debridement and may predispose for the appearance of crown discoloration as soon as root canal sealers remain and penetrate through incisally directed tubuli.

The preservation of the occlusal surface aided in the prevention of alterations in specimen's optical properties due to removal of tooth structure, placement of restorative materials, and coronal microleakage. In addition, the placement of apical seal and the preservation of distilled water up to the specimens' height prevented the effect of other sources of variability, which cannot be properly standardized or may lead to bias, such as the solubilization of sealers and the external staining of the specimens by their leaching components.

During specimen preparation, the smear layer was not removed. The application of EDTA is mainly indicated for the removal of smear layer from the root dentine of the entire root canal system and especially of the apical third [22]. There are no available studies showing the effects of EDTA in coronal dentine of the pulp chamber during irrigation. In clinical practice, the presence of a layer of inorganic debris in the pulp chamber may exist after the removal of the excessive core root canal filling materials. This may prevent or slow the possibility for diffusion of sealer chromogenic components into the tubules due to the occlusion of dentinal tubules. The results of a previous study showed that sealer-induced coronal discoloration occurs even in the presence of smear layer [6].

Significant emphasis was given for the preservation of all specimens in a relatively humid state during the measuring process in order to prevent enamel color alterations caused by drying that lead to experimental bias. The dehydration of dental tissues and enamel, especially in laboratory and clinical conditions, leads to reversible changes in the optical properties including enhancement of reflectance properties and subsequent lightness increase [16, 23].

The sole measurement of CIE total color differences from the equation $E = [(L*)^2 + (\alpha*)^2 + (b*)^2]^{\frac{1}{2}}$ allows for a mathematical comparison. Although the equation does not indicate the direction of the color differences, it clearly demonstrates the intensity of color changes which can be distinguished by the human eye. In dental science, when $\Delta E$ values are less than 1 U, then color match occurs and any


**Fig. 1** Group 1 (Roth 811). *A* Initial color appearance of crown ($t_0$). *B*, *C* Severe, perceptible discoloration of the cervical and middle third of the crown, during the first and sixth months of examination

color differences cannot be identified by independent observers in vitro [19]. However, color determination in clinical dentistry may become complicated by adjacent anatomic structures and lighting conditions. As a result, the proposed acceptance for color matching in dentistry has been set to 3.7 U (perceptibility threshold), beyond which differences are clinically visible [15].

The results of this study are comparable with the results of one quantitative study, in which sealer-induced discoloration was examined by computer analysis of digital images [8]. After 6 months, $\Delta E$ values of the tested ZnOE-containing sealers exceeded the perceptibility threshold (6 U) [8]. In the present study, $\Delta E$ values of Roth 811 sealer exceeded the perceptibility threshold after 1 week of examination (Fig. 1). Although it is not easy to determine the exact time for the initial rise of perceptible discoloration, previous studies have shown that visible crown discoloration may appear after 7 weeks and 3 months of observation, respectively [4–6]. Our findings can be explained by the fact that the first measurement took place 1 week after sealer placement. Despite the differences in the experimental methodologies, the results of the available studies indicate that ZnOE sealers exhibit fast and severe chromogenic effects in crowns [4–6]. The chromogenic potential of ZnOE sealers has been attributed to the unstable chemical bond between ZnO and eugenol [24]. Even after the end of the setting reaction, eugenol release leads to self-oxidation and becomes darker with time [25]. Moreover, ZnOE sealers present increased solubility [26, 27]. This type of crown discoloration has been characterized as chemical discoloration [24]. Clinicians should be aware of the presence of ZnOE sealer remnants, especially in the anterior esthetic zone. Thorough debridement of the pulp chamber is essential to prevent or to reduce the possibility of discoloration rise.

AH-26 sealer did not induce chromatic alterations that exceeded the perceptibility threshold. Our results are in accordance to a previous report, in which $\Delta E$ values ranged from 2.2 to 3.3 U during the first 6 months of examination [8]. However, the visual assessment of crowns in Group 2 revealed localized grayish discoloration in the cervical third. This macroscopic finding is in accordance with previous studies, which investigated crown discoloration by means of visual assessment [5, 6]. Based on the current instrumental measurements, this finding did not affect the overall chromatic appearance of the crown (Fig. 2). In the first generation of silver-containing AH-26 sealer, the chromogenic potential was attributed to the release of silver ions during and following setting reaction [5]. Despite the manufacturers' claims that new-generation epoxy-based sealers are silver-free, it is obvious that the risks of potential discoloration have not been overcome yet.

Crown discoloration induced by GuttaFlow and Epiphany SE sealers has not been investigated before. GuttaFlow is a polyvinylosiloxane-based sealer, in which gutta-percha particles are incorporated for better consistency with gutta-percha core material and adaptation to the canal walls [28]. Gutta-percha is capable of inducing mild pinkish discoloration after 15 weeks of visual assessment by experienced observers [4]. The measurement of color differences induced by gutta-percha showed that $\Delta E$ values ranged from 2.2 to 2.7 U after 6 months of examination [8]. Despite the methodological differences, our study confirmed the previous report and the results showed that neither the incorporated gutta-percha nor the polyvinylosiloxane matrix of GuttaFlow induced perceptible color differences.

Epiphany SE has been manufactured as a new version of the first-generation Epiphany sealer, with improved


**Fig. 2** Group 2 (AH26). *A* Initial color appearance of crown ($t_0$). *B* Mild decrease of lightness of the cervical third of the crown in the first month of examination. *C* Intense local grayish discoloration of the cervical third of the crown during the sixth month of examination (*blue arrow*)

physicochemical and handling properties [29, 30]. Based on a theoretical concept, it was expected that humidity could have led to setting contraction and sealer color changes, affecting the overall tooth color. On the contrary, the polymerized resinous matrix did not present any staining potential, whereas the induced chromatic alterations were not perceptible. Epiphany SE was the only sealer that led to a different pattern of total color changes in comparison with the increasing tendency observed in Groups 1–3. More specifically, $\Delta E$ values presented an initial short-term increase up to the first month and then dropped in levels equal to the control group. This observation may be attributed to alterations in specimens' optical properties due to the physical presence of the sealer in the internal dentinal surface and possible interactions of the resinous matrix with dentin. However, this statement requires further investigation.

Within the limitations of the experimental study, all tested sealers induced varying chromatic alterations. In the rejection of the null hypothesis, Roth 811 sealer induced fast and severe discoloration and exceeded the perceptibility threshold 1 week after sealer placement. Therefore, any sealer remnants should be removed from the pulp chamber after root canal obturation and before placement of a final restoration. AH 26 induced moderate discoloration that tended to reach the perceptibility threshold after 6 months. GuttaFlow and Epiphany SE sealers posed the least risk for potential staining effects. Apart from basic properties such as biocompatibility and good sealing ability, chromogenic potential may also play an important role in selecting proper root canal sealers. The proposed experimental model is appropriate for the investigation of the chromogenic potential of interacting endodontic biomaterials, including root canal irrigants, medicaments, and newly developed root canal filling and repair materials.

**Acknowledgments** The authors would like to thank Professor Palaghias George for the kind concession of the laboratory equipment in the Department of Basic Dental Sciences, School of Dentistry, Aristotle University of Thessaloniki, Greece.

**Conflict of interest** None.

## References

1. Dugas NN, Lawrence HP, Teplitsky P, Friedman S (2002) Quality of life and satisfaction outcomes of endodontic treatment. J Endod 28:819–827
2. Watts A, Addy M (2001) Tooth discoloration and staining: a review of the literature. Br Dent J 190:309–316
3. Plotino G, Buono L, Grande NM, Pameijer CH, Somma F (2008) Nonvital tooth bleaching: a review of the literature and clinical procedures. J Endod 34:394–407
4. van der Burgdt TP, Plasschaert AJM (1985) Tooth discoloration induced by dental materials. Oral Surg Oral Med Oral Pathol 60:666–669
5. van der Burgdt MTP, Plasschaert AJM (1986) Tooth discoloration induced by endodontic sealers. Oral Surg Oral Med Oral Pathol 61:84–89
6. Parsons JR, Walton RE, Ricks-Williamson L (2001) In vitro longitudinal assessment of coronal discoloration from endodontic sealers. J Endod 27:699–702
7. Davis M, Walton RE, Rivera EM (2002) Sealer distribution in coronal dentin. J Endod 28:464–466
8. Partovi M, Al-Havvaz AH, Soleimani B (2006) In vitro computer analysis of crown discoloration from commonly used endodontic sealers. Austr Endod J 32:116–119
9. Joiner A, Hopkinson I, Deng Y, Westland S (2008) A review of tooth color and whiteness. J Dent 36(Suppl 1):S2–S7
10. Sproull RC (1973) Colour matching in dentistry. Part II. Practical applications of the organization of colour. J Prosthet Dent 29:556–566
11. Paul S, Peter A, Pietrobon N, Hammerle CHF (2002) Visual and spectrophotometric analysis of human teeth. J Dent Res 81:578–582
12. Karamouzos A, Papadopoulos MA, Kolokithas G, Athanasiou AE (2007) Precision of in vivo spectrophotometric colour evaluation of natural teeth. J Oral Rehabil 34:613–621
13. Da Silva JD, Park SE, Weber HP, Ishikawa-Nagai S (2008) Clinical performance of a newly developed spectrophotometric system on tooth color reproduction. J Prosthet Dent 99:361–368
14. CIE (Commision Internationale d' Eclairage) (1978) Recommendations on uniform colour spaces, colour difference equations, psychometric colour terms. Supplement 2 of CIE Publication No.15 E.1.3.1. Central Bureau de la CIE, Paris
15. Johnston WM, Kao EC (1989) Assessment of appearance match by visual observation and clinical colorimetry. J Dent Res 68:819–822
16. Brodbelt RHW, O'Brien WJ, Fan PL, Frazer-Dib JG, Yu R (1981) Translucency of human dental enamel. J Dent Res 60:1749–1753
17. Intrayan A (2008) Medical biostatistics. Chapman & Hall/CRC Press, Boca Raton
18. Brown H, Prescott R (2003) Applied mixed models in medicine. Wiley, West Sussex
19. Seghi RR, Hewlett ER, Kim J (1989) Visual and instrumental colorimetric assessments of small color differences on translucent dental porcelain. J Dent Res 68:1760–1764
20. ten Bosch JJ, Coops JC (1995) Tooth color and reflectance as related to light scattering and enamel hardness. J Dent Res 74:374–380
21. Paul S, Peter A, Pietrobon N, Hammerle CHF (2002) Visual and spectrophotometric analysis of human teeth. J Dent Res 81:578–582
22. Kouvas V, Liolios E, Vassiliadis L, Parissis-Mesimeris S, Boutsioukis A (1998) Influence of smear layer on depth of penetration of three endodontic sealers: a SEM study. Endod Dent Traumatol 14:191–195
23. Russel MD, Gulfraz M, Moss BW (2000) In vivo measurement of colour changes in natural teeth. J Oral Rehabil 27:786–792
24. Wilson AD, Batchelor RF (1970) Zinc oxide-eugenol cements. Study of erosion and disintegration. J Dent Res 49:593–598
25. Weinberg JE, Rabinowitz RL, Zanger M, Gennaro AF (1972) $^{14}$C-eugenol: I. Synthesis, polymerization and use. J Dent Res 51:1055–1061

26. McMichen FRS, Pearson G, Rahbaran S, Gulabivala K (2003) A comparative study of selected physical properties of five root canal sealers. Int Endod J 36:629–635
27. Schäfer E, Zandbiglari T (2003) Solubility of root canal sealers in water and artificial saliva. Int Endod J 36:660–669
28. Elayouti A, Achleiter C, Löst C, Weiger R (2005) Homogenicity and adaptation of a new gutta-percha paste to root canal walls. J Endod 31:687–690
29. Resende LM, Rachhed-Junior FJ, Versiani MA et al (2009) A comparative study of physicochemical properties of AH Plus, Epiphany and Epiphany SE root canal sealers. Int Endod J 42:785–793
30. Marin-Bauza GA, Rached-Junior FJ, Souza-Gabriel AE, Sousa-Neto MD, Miranda CE, Silva-Sousa YT (2010) Physicochemical properties of methacrylate resin-based root canal sealers. J Endod 36:1531–1536