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Effects of different discoloration challenges and whitening treatments on dental hard tissues and composite resin restorations

Xiaoyi Zhao[a,b,1], Filippo Zanetti[c,1], Lin Wang[a,b], Jie Pan[b], Shoaib Majeed[c], Hans Malmstrom[a], Manuel C. Peitsch[c], Julia Hoeng[c], Yanfang Ren[a,*]

[a] University of Rochester Eastman Institute for Oral Health, 625 Elmwood Ave., Rochester, NY, 14620, USA
[b] Peking University School of Stomatology, 22 Zhongguanchun South Ave., Beijing, 100081, China
[c] PMI R&D, Philip Morris Products S.A., Quai Jeanrenaud 5, CH-2000, Neuchâtel, Switzerland

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ABSTRACT

Objectives: To compare the relative effects of cigarette smoke (CS), electronic cigarette (EC), red wine, coffee, and soy sauce on the color of enamel, dentin, and composite resin restorations, as well as the effects of whitening treatments.

Methods: Seventy premolars with composite restorations were exposed to CS, EC aerosol (a novel EC device with MESH™ technology [P4M3 version 1.0, Philip Morris International]), red wine, coffee, and soy sauce for 56 min/day for 15 days. Two whitening sessions with 6% and 35% hydrogen peroxide (H_2O_2) were performed on the exposed samples. Teeth exposed to CS and EC aerosol were also brushed with whitening toothpaste for 3 weeks. Color match of resin restorations was assessed, and color changes were compared after exposure and after whitening treatments.

Results: Discolorations in enamel, dentin, and composite resin were observed in the order of red wine > CS > soy sauce > coffee > EC. Color mismatch between enamel and resin restorations occurred only in red wine and CS groups. Brushing with whitening toothpaste removed discoloration caused by EC aerosol; H_2O_2 treatments were necessary to eliminate discolorations caused by coffee and soy sauce. Discolorations of dentin and resin restorations could not be completely removed by whitening treatments, and color mismatch remained in teeth exposed to red wine and CS.

Conclusion: Red wine and CS cause significant tooth discoloration and color mismatch in enamel and resin restorations that are not reversible by whitening treatments. Tooth discoloration associated with EC aerosol was minimal and could be removed by brushing with whitening toothpaste.

Clinical significance: Red wine drinkers and cigarette smokers have increased risks for tooth discoloration and color mismatch between enamel and composite resin restorations. Whitening treatments may not be effective in correcting the color mismatch. Tooth discoloration associated with EC aerosol is minimal.

1. Introduction

Whitening of discolored teeth is one of the most commonly performed dental procedures worldwide, as whiter teeth are esthetically desirable in most cultures [1,2]. Tooth discoloration is often associated with pigments in food, beverages, and other substances that come into frequent contact with dental hard tissues in the oral cavity. Use of tobacco products is also a known risk factor for tooth discoloration.

The natural color of the human tooth crown is mostly white, with slight yellow and minute red tints that reflect the color of the dentin below the transparent enamel [3]. Many beverages contain natural or artificial pigments that can affect the color of dental hard tissues. Beverages with an intense dark color, such as coffee and red wine, are regarded as common risk factors for tooth discoloration [4]. Soy sauce, a commonly used condiment, may also negatively affect tooth color [5].

Cigarette smoke (CS) may similarly affect tooth color, and smokers are more likely to exhibit tooth discoloration than non-smokers [6–8]. Products that heat rather than combust tobacco have been shown to exert significantly less impact on the colors of teeth and composite resin restorations, presumably owing to the absence of tar [9]. Electronic cigarettes (EC) deliver nicotine by heating and aerosolizing a glycerin-based nicotine solution [10]. Although nicotine is colorless, it turns yellow when

* Corresponding author.
E-mail address: yanfang_ren@urmc.rochester.edu (Y. Ren).
[1] Contributed equally to the study

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oxidized; as nicotine can penetrate dental hard tissues, it is possible that EC aerosol may affect tooth color. A few studies have investigated the effect of EC aerosol on the color of bovine enamel, with inconsistent findings. EC aerosol was reported to significantly alter the color of bovine enamel after 200 puffs in a recent study [11], while no adverse effect on color was reported after 5 days of use in another [12]. Both studies used short exposure durations and bovine enamel discs ground flat. EC effects on intact human teeth are not known, especially over a longer period.

Although CS, EC aerosol, red wine, coffee, and soy sauce have all been shown to affect tooth color, few studies have investigated their relative effects. Red wine was found to cause more severe tooth discoloration than coffee [4], and CS caused more surface staining on bovine enamel than EC aerosol [12]. Most of these studies reported the effects of experimental discoloration challenges without taking into consideration the influence of daily tooth brushing. Unlike surface stains, which can be removed with daily brushing and regular toothpaste, internalized discoloration that persists after tooth brushing is of true clinical significance because of its impact on esthetics and potential desire for clinical intervention. Therefore, it would be of interest to determine to what extent daily brushing affects tooth discoloration caused by various substances and whether tooth-whitening treatments are necessary to reverse the discoloration in dental hard tissues and esthetic dental restorations.

Most tooth discoloration studies have focused on dental enamel, which is normally exposed in the oral cavity and contributes most to the esthetic perception of human teeth. There is increasing interest in the capacity of dental restorative materials to resist discoloration, as color stability is an important factor in treatment success for dental restoration in the esthetic zone. Multiple studies have shown that the factors that affect tooth color may exert similar effects on esthetic restorative materials, especially composite resins [13–15]. As composite resins differ greatly from dental enamel in composition and in chemical and physical properties, they are likely affected to a different degree when exposed to the same discoloration challenges, which may result in color mismatch and treatment failure [9]. For example, CS has been shown to cause more discoloration in composite resin than in enamel in teeth with Class V restorations, resulting in eventual color mismatch that became clinically unacceptable [9]. In addition, dentin exposure is a common finding in the general population, both in individuals with excellent dental hygiene and in patients with periodontal diseases [16,17]. As dentin contains more organic substances than enamel and has communicating tubular structures, it may be prone to discoloration and compromise facial esthetics. Our previous study reported that the effects of CS differed in dentin and in enamel and composite resins [9]. It may be worthwhile to investigate whether various discoloration challenges produce similar effects in a model that allows direct comparison of color changes in enamel, dentin, and composite resin restorations.

In the present study, we hypothesize that CS, EC aerosol, red wine, coffee, and soy sauce affect the color of dental hard tissues and composite resins to different degrees and thus induce color mismatch of esthetic dental restorations, and that daily brushing and tooth-whitening treatments reduce or reverse the discolorations of dental enamel, dentin, and esthetic dental restorations. The objectives of the present study were therefore to test the relative effects of CS, EC aerosol, red wine, coffee, and soy sauce on the color of human enamel, dentin, and composite resin restorations; to examine if regular tooth brushing affects the outcomes of various exposures on dental hard tissues and composite resin restorations; to determine whether professional whitening treatments with 6% and 35% hydrogen peroxide (H_2O_2) could reverse the discoloration induced by these substances; and to test whether brushing with consumer whitening toothpaste would reverse tooth discoloration associated with CS and EC aerosol.

2. Materials and methods

2.1. Tooth collection and specimen preparation

Human premolars extracted for orthodontic reasons were collected fresh from oral surgery clinics and sterilized with ethylene oxide overnight for 12 h before use. Tooth collection was conducted in accordance with the guidelines at the University of Rochester, Eastman Institute for Oral Health and to those at the Peking University School of Stomatology (approval number: PKUSSIRB-201839149). To allow initial color match with selected composite resin restorative materials, only teeth matching the A2 shade on a VITA Classical shade guide (VITA Zahnfabrik, Bad Säckingen, Germany) were selected, and those with discoloration and enamel defects were excluded.

Details of specimen preparations have been reported elsewhere [9]. Briefly, Class V cavities, 3 mm × 5 mm in diameter and 2 mm deep, were first prepared in all teeth with the apical wall extended 1.0 mm into the dentin below the cemento-enamel junction, then restored with a nanofilled universal composite resin (shade A2 enamel, Filtek Supreme Ultra, 3 M ESPE) and Scotchbond universal adhesive (3 M ESPE), light cured, and polished with Sof-Lex discs in sequences of 4 from coarse to superfine grit following the manufacturer's instructions. Cementum below the Class V restorations on the root surface was intentionally removed using polishing discs to expose the underlying dentin. A total of 70 teeth were prepared and restored.

Digital photographs of the specimens were taken at baseline and after each stage of experiments with a digital single-lens reflex camera (Canon T6i with a Sigma 105 mm f/2.8 macro lens) mounted on a photo stand with four 60 W daylight bulbs oriented at 45° angles.

2.2. VITROCELL® 3R4F CS and MESH Classic Tobacco aerosol exposures

Group I (20 samples) was exposed to CS from 3R4F reference cigarettes (Kentucky Tobacco Research & Development Center; University of Kentucky, Lexington, KY, USA) using a 30-port carousel smoking machine (SM2000; Philip Morris International). Group II (20 samples) was exposed to the aerosol generated from the *MESH* Classic Tobacco liquid using a novel EC device with *MESH* technology (P4M3 version 1.0, Philip Morris International) and single-programmable syringe pumps. The number of puffs corresponded to the duration of the exposure. An approximate 75-min exposure to the *MESH* Classic Tobacco aerosol, equivalent to around 300 puffs, corresponded to nearly a whole cartridge each day of exposure. A 56-min exposure to the 3R4F CS, equivalent to 224 puffs, corresponded to smoking 20 cigarettes each day of exposure. A detailed description of the exposure design appears in the Supplementary Materials and Methods.

2.3. Exposures to coffee, red wine, and soy sauce

Group III (10 samples) was exposed to a coffee solution prepared with 1.8 g coffee (Nescafe Rich Blend, Vevey, Switzerland; pH 5.38) dissolved in 150 mL of boiling water and cooled to room temperature. Group IV (10 samples) was exposed to red wine (Reserve du Vigneron Rouge 2010, Les Caves du Mistral, France; 12% alcohol by volume, pH 3.36), and Group V (10 samples) was exposed to soy sauce (Golden Lion Soy Sauce, Beijing Liubijv Food Company, Tianjin, China; pH 4.13). Specimens were mounted on a small acrylic base to allow the buccal surfaces with Class V composite restorations to face upwards and placed into 24-well cell culture plates, with each well containing a single specimen. Two milliliters of coffee, red wine, and soy sauce were then added to the cell culture wells, and the specimens were fully submerged in the liquids. The specimens were exposed for 56 min per day for 15 days at 22 ± 1 °C.

The exposure experiment was conducted for 15 days (Fig. 1). The enamel, dentin, and composite restoration specimens were brushed daily in the morning with 20 strokes each under 200-*g* pressure at a frequency of approximately 120 strokes per minute using the American Dental Association reference toothbrush (Chicago, IL, USA) and a toothpaste containing hydrated silica abrasives (Crest Regular Cavity Protection, Proctor & Gamble Inc., Cincinnati, OH, USA). This vigorous brushing protocol ensured that all colorants adsorbed onto the tissue or material surfaces were removed.

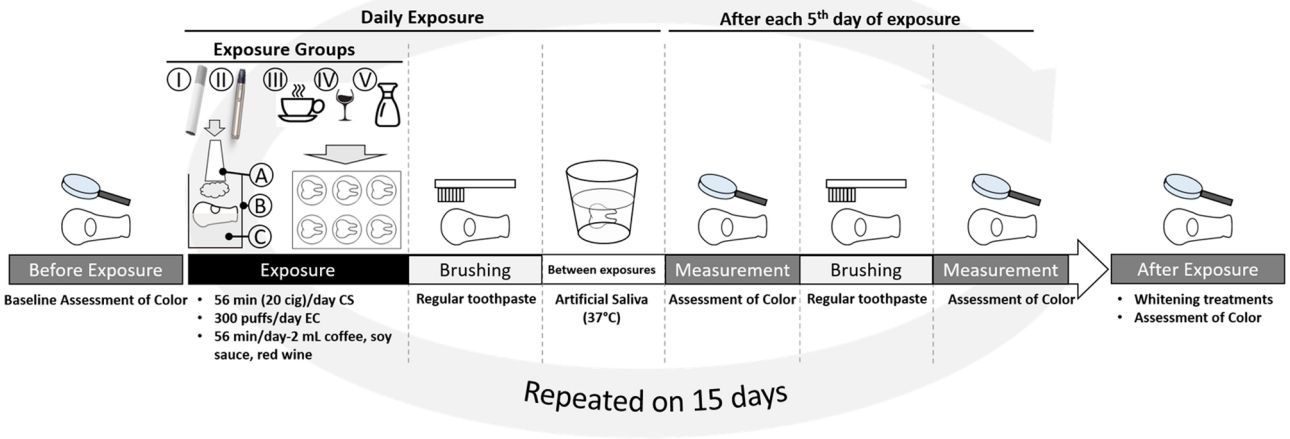

Fig. 1. Study design.
Seventy human premolar teeth were measured for color at baseline. The specimens were exposed in a VITROCELL® 24/48 exposure system to 3R4F CS or *MESH* Classic Tobacco aerosol for 15 days, 20 cigarettes, or 300 puffs of EC aerosol or 56 min of coffee, red wine, or soy sauce per day. Between exposures, the specimens were stored in artificial saliva. A brushing protocol with regular toothpaste was applied the day after each exposure. A whitening session was performed at the completion of the exposure period; color was assessed before and after regular brushing and after each session of the whitening protocols. A, VITROCELL® trumpet; B, plate well; C, mold to allocate premolar teeth.

Specimens were placed in artificial saliva (composition described by Zhao et al. [18]) at 37 °C and pH 7 between exposures. Artificial saliva was freshly prepared and changed every day during the experiment.

2.4. Color assessments

The color of the enamel, root dentin, and composite resin restorations was assessed in the Commission Internationale de l'Éclairege L*a*b* (CIE Lab) color space using an Olympus CrystalEye® dental spectrophotometer (Olympus, Tokyo, Japan) as described previously [9]. The CIE Lab system is a chromatic value color space that measures the value and chroma on 3 coordinates:

- L*: the lightness of the color measured from black (L* = 0) to white (L* = 100)
- a*: the color in the red (a* > 0) and green (a* < 0) coordinates
- b*: the color in the yellow (b* > 0) and blue (b* < 0) coordinates [19]

Color was assessed before the start of exposure (baseline), after each 5 days of exposure, and both before and after brushing with regular toothpaste. Color variations from baseline (ΔE) were calculated from the L*, a*, and b* values for each specimen using the formula $\Delta E = \{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2\}^{1/2}$. As ΔE value was calculated as the square root of the sum of the squared differences in CIE Lab color space, it was always a positive value, independent of the direction of the color shifts. For the purpose of the present study, we designated the ΔE value as negative if the substrates became more discolored than baseline values (*i.e.*, when lightness [L*] decreased and redness [a*] or yellowness [b*] increased) after the exposures and as positive if the substrates became less discolored than baseline values (*i.e.*, when lightness [L*] increased and redness [a*] or yellowness [b*] decreased) after the tooth-whitening treatments described below.

Samples were evaluated by two investigators using the modified U.S. Public Health Service (USPHS) criteria described by Wilson and colleagues [20] for color matching, marginal discoloration, marginal integrity, and surface texture. Teeth surfaces were kept moist at all time during color assessments and the specimens were immersed in artificial saliva immediately after each measurement.

2.5. Whitening treatments with H_2O_2 and whitening toothpaste

2.5.1. Treatments with take-home whitening gel with 6% H_2O_2

Ten specimens from each group were treated with a commercial tooth-whitening gel with 6% H_2O_2 (Beyond Core White, Beyond International Inc., Stafford, TX, USA). Each specimen was treated for 30 min per day for 8 days, for a total of 240 min. The CIE Lab colors of enamel, dentin, and composite resin restorations were assessed after 30, 120, and 240 min of whitening treatment.

2.5.2. Treatments with in-office whitening gel with 35% H_2O_2

Ten specimens from Groups I (CS) and II (*MESH* Classic Tobacco aerosol) and all specimens from Groups III (coffee), IV (red wine), and V (soy sauce) that were treated with 6% H_2O_2 were further treated with an in-office tooth-whitening product containing 35% H_2O_2 (Beyond Max5, Beyond International Inc.) Each specimen was treated for 30 min per session for a total of 2 sessions following the manufacturer's instructions. The CIE Lab colors of enamel, dentin, and composite resin restorations were assessed after 30 and 60 min of whitening treatment.

2.5.3. Brushing with tooth-whitening toothpaste

To test whether tooth-whitening toothpaste alone could effectively remove discolorations caused by CS or *MESH* Classic Tobacco aerosol, 10 specimens from Groups I and II were brushed with a tooth-whitening toothpaste for consumer use (Crest 3D White, Proctor & Gamble). Each specimen was brushed for 140 strokes per week under 200 *g* pressure, for a total of 3 weeks and 420 strokes. The CIE Lab colors of enamel, dentin, and composite resin restorations were assessed after 1, 2, and 3 weeks of brushing with the whitening toothpaste.

2.6. Statistical analysis

The primary outcome measure was the overall color difference (ΔE) of enamel, dentin, and composite resin restorations before and after exposure to 3R4F CS, *MESH* Classic Tobacco aerosol, coffee, red wine, and soy sauce. Based on our previous study, a sample of 10 per group would allow 90% power at an alpha level of 0.01 to detect the expected differences between groups. Differences in ΔE among the 5 groups were compared in enamel, dentin, and composite resins at various stages of the experiment and after whitening treatments using analysis of variance and post hoc Fisher's least significant difference (LSD) test. Color changes before and after exposure in

each group and in teeth exposed to CS or *MESH* Classic Tobacco aerosol after brushing with a whitening toothpaste were compared using paired *t*-tests. Only descriptive analysis was used for the USPHS criteria for color match, marginal discretion, marginal integrity, and surface texture. All statistical analyses were based on 2-tailed tests at an alpha level of 0.05. Bonferroni corrections were applied to all statistical analyses to adjust for potential errors associated with multiple comparisons.

3. Results

3.1. Baseline assessments

At baseline, there were no differences between groups in the color of enamel, dentin, and composite resin restorations measured in the CIE Lab color space, except the redness of dentin and yellowness of composite resin restorations (Supplementary Tables 1–3). These differences were small in magnitude and were likely due to random variations in the color of dentin and composite resins. There was no color mismatch between enamel and composite resin restorations, evaluated using the modified USPHS criteria (Supplementary Tables 4–8).

3.2. Effects of exposures on enamel, dentin, and composite resin restorations

3.2.1. Effects of regular tooth brushing on discoloration

There were marked discolorations in enamel, dentin, and composite resin restorations after exposure to CS, coffee, red wine, and soy sauce (Fig. 2). The *MESH* Classic Tobacco group exhibited less discoloration than the other groups. Brushing with regular toothpaste removed large

Fig. 2. Tooth discoloration after 5, 10, and 15 days of exposure to cigarette smoke, electronic cigarette aerosol, coffee, red wine, or soy sauce before and after brushing. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).
Representative pictures of human premolar teeth restored with composite resins. Images were taken after each 5 days of exposure to cigarette smoke, electronic cigarette aerosol (*MESH* Classic Tobacco), coffee, red wine, or soy sauce, before and after brushing with regular toothpaste.

Table 1
ΔE values following 15 days of exposure.

		5 days	10 days	15 days
Enamel	**CS**	-4.71 ± 1.72^{a}	-6.72 ± 1.98^{a}	-8.37 ± 2.33^{a}
	EC aerosol	-0.62 ± 0.23^{b}	-1.57 ± 0.54^{b}	$-2.27 \pm 0.53^{b,c}$
	Coffee	-1.74 ± 0.98^{c}	$-2.39 \pm 0.86^{b,d}$	$-3.43 \pm 1.21^{c,e}$
	Red wine	-6.76 ± 1.21^{d}	-10.79 ± 1.31^{c}	-16.28 ± 2.22^{d}
	Soy sauce	-1.90 ± 1.29^{c}	-3.15 ± 1.25^{d}	-4.37 ± 1.42^{e}
	p	$<$ **0.0001**	$<$ **0.0001**	$<$ **0.0001**
Dentin	**CS**	-13.23 ± 5.24^{a}	-18.17 ± 4.38^{a}	-21.44 ± 4.44^{a}
	EC aerosol	-1.00 ± 0.57^{b}	-2.16 ± 0.85^{b}	-2.81 ± 0.91^{b}
	Coffee	-13.20 ± 2.99^{a}	-19.88 ± 3.65^{a}	-21.84 ± 4.33^{a}
	Red wine	-35.84 ± 6.80^{c}	-44.36 ± 8.09^{c}	-50.27 ± 7.63^{c}
	Soy sauce	-20.84 ± 6.97^{d}	-24.86 ± 6.94^{d}	-28.63 ± 7.24^{d}
	p	$<$ **0.0001**	$<$ **0.0001**	$<$ **0.0001**
Composite resin	**CS**	-8.69 ± 2.98^{a}	-11.29 ± 3.94^{a}	-13.73 ± 4.18^{a}
	EC aerosol	-0.77 ± 0.41^{b}	-1.57 ± 0.76^{b}	-2.37 ± 1.00^{b}
	Coffee	-4.97 ± 0.79^{c}	$-6.05 \pm 0.84^{c,d}$	-7.08 ± 0.92^{c}
	Red wine	-8.21 ± 1.13^{a}	$-10.58 \pm 0.67^{a,e}$	-13.17 ± 1.08^{a}
	Soy sauce	-4.74 ± 1.23^{c}	$-6.27 \pm 1.66^{d,f}$	-7.15 ± 1.63^{c}
	p	$<$ **0.0001**	$<$ **0.0001**	$<$ **0.0001**

Analysis of variance and post hoc Fisher's LSD test with Bonferroni corrections. ΔE values (means $\pm$ SD) are designated negative to indicate increased discoloration from the baseline. Different letters in groups in the same column denote statistical significant difference (post hoc Fisher's least significant difference test); when the same letter is present in different groups from the same column it denotes that these groups are not statistically different. CS, cigarette smoke; EC, electronic cigarette (*MESH* Classic Tobacco).

amounts of the colorants adsorbed onto the surfaces of the substrates, especially for CS and soy sauce, as indicated by significant differences in ΔE values before and after brushing (Supplementary Fig. 1). Overall, discoloration of enamel was insignificant after 5–10 days of exposure and tooth brushing in the *MESH* Classic Tobacco and coffee groups ($\Delta E < 2.4$, Table 1). In general, discoloration of dentin and composite resin restorations was more readily discernible to the naked eye both before and after brushing than discoloration of enamel throughout the experimental period and across the exposures. As we were focused on discolorations that could not be readily removed mechanically, only measurements of CIE Lab colors after brushing were used in the analyses hereafter.

3.2.2. Effects of exposures on enamel, dentin, and composite resins

After 15-day exposures, dental enamel, dentin, and composite resin restorations exhibited overall trends for decreases in lightness and increases in redness and yellowness in all groups. The exposures had differing effects on components of the CIE Lab color space (Supplementary Tables 1–3 and Supplementary Fig. 2). Red wine affected the lightness and redness values of enamel and dentin to the greatest extent, while CS and *MESH* Classic Tobacco aerosol affected the colors of enamel, dentin, and composite resins to varying extents. CS caused a significant decrease in lightness and significant increases in redness and yellowness in enamel, dentin, and composite resin restorations (Supplementary Tables 1–3 and Supplementary Fig. 2). A much smaller color change was observed in the *MESH* Classic Tobacco group, and after 15 days, the CIE Lab values were statistically significantly different from baseline values in lightness, redness, and yellowness for dentin and composite restorations but only in lightness and yellowness for enamel ($p < 0.001$, paired *t*-test).

3.2.3. Relative effects of exposures

In general, red wine caused the greatest discoloration ($\Delta E = 26.6 \pm 17.7$), followed by CS ($\Delta E = 14.5 \pm 6.6$), soy sauce ($\Delta E = 13.4 \pm 11.8$), coffee ($\Delta E = 10.8 \pm 8.5$), and *MESH* Classic Tobacco aerosol ($\Delta E = 2.5 \pm 0.8$) (average ΔE values between enamel, dentin, and composite resin). As indicated by the large standard deviations (SD) of the mean ΔE values, we observed that the effects of red wine, CS, coffee, and soy sauce varied greatly by substrate, with dentin exhibiting much larger changes in color than enamel (Table 1). *MESH* Classic Tobacco aerosol affected the colors of enamel, dentin, and composite resins more uniformly and to a lesser extent, with ΔE values under 3.0 for all 3 substrates after 15 days of exposure (Table 1).

3.2.3.1. Color changes in enamel.

Red wine had the greatest effects on enamel ($\Delta E = 16.3$), followed by CS ($\Delta E = 8.4$), soy sauce ($\Delta E = 4.4$), and coffee ($\Delta E = 3.4$). *MESH* Classic Tobacco aerosol had the least effect on enamel ($\Delta E = 2.3$) (Table 1 and Supplementary Fig. 3).

3.2.3.2. Color changes in dentin.

Red wine also had the greatest effects on dentin color ($\Delta E = 50.3$), followed by soy sauce ($\Delta E = 28.6$), coffee ($\Delta E = 21.8$), and CS ($\Delta E = 21.4$). *MESH* Classic Tobacco aerosol had the least effect on dentin ($\Delta E = 2.8$) (Table 1 and Supplementary Fig. 3).

3.2.3.3. Color changes in composite resin.

CS ($\Delta E = 13.8$) and red wine ($\Delta E = 13.2$) had greater effects than soy sauce ($\Delta E = 7.2$) and coffee ($\Delta E = 7.1$) on the color of composite resin restorations. *MESH* Classic Tobacco aerosol had the least effect on dental composite resins ($\Delta E = 2.4$) (Table 1 and Supplementary Fig. 3).

3.3. Effects of exposures on color match of composite resin restorations

Using the modified USPHS criteria for evaluation of the composite resin restorations (see Supplementary Materials for detailed descriptions), all Class V restorations were rated Alpha at baseline for color match, marginal discoloration, marginal integrity, and surface texture in the 5 comparison groups (Supplementary Tables 4–8). After a 15-day exposure, the color match deteriorated, and marginal discoloration increased in teeth exposed to red wine, CS, coffee, and soy sauce but not in teeth exposed to *MESH* Classic Tobacco aerosol. Color match was rated Charlie in all teeth in the red wine group, Charlie in 2 teeth and Bravo in 8 teeth in the CS group, and Bravo in all teeth in the coffee and soy sauce groups. Marginal discoloration was rated Bravo in all teeth in the red wine and CS groups, Alpha in 1 tooth and Bravo in 9 teeth in the coffee group, and Alpha in 2 teeth and Bravo in 8 teeth in the soy sauce group. Alpha rating was maintained for color match and marginal discoloration in the *MESH* Classic Tobacco group. Marginal integrity and surface texture were not affected in any group.

Table 2
ΔE values following whitening treatments with 6% and 35% H_2O_2.

		6% H_2O_2 1 day	6% H_2O_2 4 days	6% H_2O_2 8 days	35% H_2O_2 30 min	35% H_2O_2 60 min
Enamel	**CS**	-6.41 ± 2.27^{a}	-4.60 ± 2.53^{a}	-3.68 ± 2.52^{a}	4.45 ± 1.93^{a}	5.37 ± 2.63^{a}
	EC aerosol	-1.19 ± 0.65^{b}	1.49 ± 1.07^{b}	1.84 ± 1.00^{a}	NA	NA
	Coffee	-2.43 ± 1.20^{b}	-1.76 ± 0.95^{b}	2.02 ± 1.44^{a}	$6.70 \pm 1.42^{a,b}$	9.85 ± 1.56^{b}
	Red wine	-8.27 ± 1.54^{a}	-4.93 ± 1.47^{a}	-4.29 ± 1.13^{a}	5.35 ± 2.32^{a}	$8.53 \pm 2.52^{a,b}$
	Soy sauce	-2.91 ± 1.27^{b}	$-2.75 \pm 0.77^{a,b}$	3.97 ± 1.66^{a}	8.79 ± 1.40^{b}	11.52 ± 1.73^{b}
	p	**< 0.0001**	**< 0.0001**	**NS**	**< 0.0001**	**< 0.0001**
Dentin	**CS**	-19.43 ± 4.87^{a}	-17.91 ± 4.91^{a}	-16.75 ± 4.86^{a}	-7.58 ± 2.73^{a}	-5.97 ± 1.77^{a}
	EC aerosol	-1.83 ± 0.92^{b}	-1.12 ± 0.79^{b}	1.13 ± 0.46^{b}	NA	NA
	Coffee	-20.01 ± 4.24^{a}	-16.20 ± 3.64^{a}	-13.57 ± 2.91^{a}	-4.43 ± 1.75^{a}	6.03 ± 2.09^{a}
	Red wine	-40.60 ± 9.14^{c}	-34.62 ± 8.79^{c}	-30.77 ± 7.79^{c}	-18.68 ± 9.12^{b}	-10.57 ± 7.00^{a}
	Soy sauce	-24.47 ± 7.42^{a}	-16.27 ± 6.32^{a}	-12.63 ± 6.28^{a}	-5.59 ± 2.58^{a}	7.14 ± 2.46^{a}
	p	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**NS**
Composite resin	**CS**	-9.16 ± 2.91^{a}	-8.28 ± 2.78^{a}	$-7.18 \pm 2.92^{a,d}$	-3.89 ± 2.00^{a}	-3.65 ± 1.36^{a}
	EC aerosol	-1.38 ± 0.59^{b}	-1.17 ± 0.68^{b}	1.20 ± 0.57^{b}	NA	NA
	Coffee	-4.94 ± 1.09^{c}	$-3.32 \pm 0.99^{b,c}$	$-2.25 \pm 0.76^{b,c}$	-1.56 ± 0.56^{b}	2.06 ± 0.55^{a}
	Red wine	-8.18 ± 0.92^{a}	$-6.42 \pm 0.53^{a,c}$	-5.76 ± 0.63^{d}	-3.91 ± 0.77^{a}	-3.46 ± 0.82^{a}
	Soy sauce	$-6.12 \pm 1.68^{a,c}$	-5.00 ± 1.91^{c}	$-4.50 \pm 2.04^{c,d}$	$-3.78 \pm 1.91^{a,b}$	4.07 ± 1.97^{a}
	p	**< 0.0001**	**< 0.0001**	**< 0.0001**	**NS**	**NS**

Analysis of variance and post hoc Fisher's LSD test with Bonferroni corrections. ΔE values (means ± SD) are designated negative to indicate increased discoloration from the baseline and positive to indicate decreased discoloration from the baseline. Different letters in groups in the same column denote statistical significant difference (post hoc Fisher's least significant difference test); when the same letter is present in different groups from the same column it denotes that these groups are not statistically different. CS, cigarette smoke; EC, electronic cigarette (*MESH* Classic Tobacco); NA, not applicable; NS, not significant.

3.4. Effects of tooth-whitening treatments

3.4.1. Effects of whitening treatment with 6% H_2O_2

After 8 days of 30-min treatment with 6% H_2O_2, enamel, dentin, and composite restorations recovered their original colors completely in the *MESH* Classic Tobacco group, but not in the CS and coffee, red wine groups (Table 2, Fig. 3, Supplementary Fig. 2 and 3, and Supplementary Tables 9–11). Though enamel color largely reverted to baseline color in the coffee and soy sauce groups, significant discolorations remained in dentin and in composite resin restorations. In general, dentin and composite resin remained darker and had more red and yellow tints than baseline values after 8 days of treatment for a total of 240 min. Red wine stains on dentin and CS stains on composite resin were the most resistant to whitening treatments (Table 2 and Supplementary Tables 10 and 11, and Supplementary Figs. 2 and 3).

3.4.2. Effects of whitening treatment with 35% H_2O_2

Teeth discolored by CS, coffee, red wine, and soy sauce were treated with 35% H_2O_2, as treatments using 6% H_2O_2 failed to completely remove the discoloration. After 2 treatment sessions with 35% H_2O_2 for a total of 60 min, discolorations were completely removed in the coffee and soy sauce groups but not in the CS and red wine groups. Red wine and CS stains could not be completely removed from dentin and composite resin restorations (Table 2, Fig. 3, Supplementary Tables 10 and 11, and Supplementary Figs. 2 and 3).

3.4.3. Effects of brushing with tooth-whitening toothpaste

As comparisons before and after brushing with regular toothpaste showed that the dark brownish stains could largely be removed from teeth in the CS group (Fig. 2), we conducted further experiments to test whether continued brushing with a whitening toothpaste marketed commercially could remove the remaining discoloration after CS exposure. As whitening with 6% H_2O_2 completely removed the discolorations of enamel, dentin, and composite resins in the *MESH* Classic Tobacco group (Table 2, Fig. 3, Supplementary Tables 9–11 and Supplementary Fig. 2 and 3), we wanted to determine whether whitening toothpaste alone could achieve a similar result. Brushing with a whitening toothpaste for 3 weeks did significantly reduce the discoloration in the CS group, with an increase in lightness and decreases in redness and yellowness in enamel, dentin, and composite restorations (Table 3, Fig. 4 and Supplementary Tables 12–14), but failed to remove the stains to a degree of clinical significance, as ΔE values in all 3 substrates remained greater than the threshold value of 3.3 that signifies discoloration (Table 3). In the *MESH* Classic Tobacco group, brushing with whitening toothpaste further increased the lightness and reduced the redness and yellowness of enamel, dentin, and composite resin, and no significant differences from baseline values in CIE Lab colors remained after 3 weeks of brushing (Table 3, Fig. 4, and Supplementary Tables 12–14).

4. Discussion

The findings of the present study indicate that red wine, CS, soy sauce, coffee, and *MESH* Classic Tobacco aerosol, in order of decreasing severity, cause discolorations in dental enamel, dentin, and composite resin restorations. Color mismatch between enamel and composite resin restorations occurred in the red wine and CS groups, as these substrates were affected to varying degrees. Brushing with a tooth-whitening toothpaste alone reversed the minor color change in the *MESH* Classic Tobacco aerosol group, but whitening treatments with 6% and 35% H_2O_2 were necessary to eliminate discolorations caused by coffee and soy sauce. Discolorations of dentin and composite resin restorations could not be completely removed, and color mismatch remained in the red wine and CS groups following the whitening treatments.

It is important to distinguish between surface stains and internalized discoloration when assessing the effects of pigmenting agents on tooth color. Surface stains on the tooth structure can be removed by simple mechanical means, such as brushing or tooth cleaning. This distinction is especially significant when evaluating the association of CS with discolorations of dental hard tissues and esthetic dental restorations. Previous studies mostly reported that CS induced enamel color change with ΔE values in the range of 20–30 after very short exposures [12,21]. We found that after 5 days of CS exposure, the ΔE values of intact human dental enamel reached 36, with lightness values at 55, redness at 11, and yellowness at 48, signifying a distinct dark brown color. After brushing with regular toothpaste, however, the corresponding ΔE value fell below 5, and lightness, redness, and yellowness values were 75, 3, and 24, respectively, reflecting a much smaller color change that is probably more representative of the *in vivo* situation. Brushing with regular toothpaste affected liquid exposure, as well, especially in the case of soy sauce. As both patients and dental professionals are likely more interested in internalized discoloration that has esthetic

Fig. 3. Effects of tooth-whitening treatments on enamel, dentin, and composite resin restorations after exposure to cigarette smoke, electronic cigarette aerosol, coffee, red wine, or soy sauce.
Representative pictures of human premolar teeth restored with composite resins. Images were taken after 15 days of exposure to cigarette smoke, electronic cigarette aerosol (*MESH* Classic Tobacco), coffee, red wine, or soy sauce, and at each time interval of whitening treatments with 6% and 35% hydrogen peroxide (H_2O_2).

significance, it is therefore advisable to include brushing with regular toothpaste in the study protocol when assessing tooth discoloration *in vitro*.

Numerous tooth-whitening toothpastes are available on the market for preventing or treating tooth discoloration. Whitening toothpastes may be more effective than regular toothpaste in stain removal through increased abrasiveness [22–24]. Brushing with tooth-whitening toothpaste alone reversed the discoloration in the group exposed to EC aerosol, especially on enamel, where a perceptible whitening effect was observed from baseline values. Though whitening toothpaste significantly reduced the surface staining in the CS group, substantial discoloration remained in enamel, dentin, and composite resins after 3 weeks of brushing. This finding is in agreement with those of previous studies, which reported that whitening toothpaste alone may not prevent enamel discoloration caused by CS, although it produced signs of enamel abrasion after 8 weeks of use [25]. Professional tooth-whitening

Table 3
ΔE values of teeth exposed to CS or EC aerosol after brushing with a whitening toothpaste.

		Before whitening toothpaste	Whitening toothpaste 1 week	Whitening toothpaste 2 weeks	Whitening toothpaste 3 weeks
Enamel	**CS**	−9.23 ± 2.90	−7.25 ± 2.57	−6.15 ± 2.54	−5.65 ± 2.19
	EC aerosol	−2.32 ± 0.82	−1.54 ± 0.73	−1.51 ± 0.76	1.52 ± 1.02
	p	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**
Dentin	**CS**	−21.81 ± 5.69	−19.41 ± 5.80	−18.11 ± 5.79	−17.12 ± 5.84
	EC aerosol	−2.62 ± 0.62	−1.63 ± 0.54	−1.09 ± 0.44	−0.99 ± 0.44
	p	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**
Composite resin	**CS**	−14.16 ± 3.15	−10.89 ± 3.54	−9.64 ± 3.74	−8.64 ± 3.62
	EC aerosol	−2.48 ± 0.91	−2.02 ± 0.90	−1.54 ± 0.94	−1.35 ± 0.90
	p	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**

t-test with Bonferroni corrections. ΔE values (means ± SD) are designated negative to indicate increased discoloration from the baseline and positive to indicate decreased discoloration from the baseline. CS, cigarette smoke; EC, electronic cigarette (*MESH* Classic Tobacco).

Fig. 4. Effects of brushing with a tooth-whitening toothpaste after exposure to cigarette smoke or electronic cigarette aerosol.
Representative pictures of human premolar teeth restored with composite resins. Images were taken after 15 days of exposure to cigarette smoke or electronic cigarette aerosol (*MESH* Classic Tobacco) and after each week of brushing with whitening toothpaste.

treatments with H_2O_2 are required to ameliorate tooth discoloration caused by CS. H_2O_2 may penetrate deep into dental hard tissues and react with chromophores to neutralize the color [26–29]. Tooth whitening by H_2O_2 has been effective in smokers [30]. We found that treatment with 6% H_2O_2 for 8 days completely removed the discoloration in dental enamel, dentin, and dental composite resins in the EC aerosol group but not in the CS group. Though enamel discoloration was eventually reversed, successive treatments with 6% and 35% H_2O_2 could not completely remove discolorations in dentin and composite restorations in the CS group. The discrepancies between dental enamel and composite resin in discoloration and in whitening efficacy indicate that color mismatch may be problematic in smokers with composite resin restorations in the esthetic zone [31].

Though the effects of CS on oral health and tooth color are well known [32–34], the effects of EC aerosol are not entirely clear. As EC aerosol contains nicotine, which turns yellow when oxidized, and most EC liquids are described as yellow, brown, or tan in color [35], EC aerosol can potentially cause tooth discoloration when inhaled through the oral cavity. A recent study reported that after 200 puffs of EC aerosol (equivalent to 1 day of use), nicotine solutions without flavoring caused mild darkening, while those with menthol or tobacco flavoring caused perceptible lightening in the color of bovine enamel [11]. The degree of color change was not clearly associated with nicotine concentrations, especially in products with flavoring. When EC aerosol exposure was extended to 5 days, however, significant color changes ($\Delta E > 3.3$) did occur, as shown in a similar study [12]. We extended the exposure to 15 days and incorporated tooth brushing into the study protocol to simulate conditions of human use; we found that *MESH* Classic Tobacco aerosol caused perceptible color changes in dental enamel, dentin, and dental composite resins, with ΔE values of 2.3–2.8. It is conceivable that the discoloration will increase with extended duration of use, as the ΔE values trended higher with time. The degree of discoloration in the *MESH* Classic Tobacco group was nonetheless much smaller than the discoloration observed in the CS group, where the ΔE values were approximately 4–10 times higher under the same conditions. The experimental cartridges EC used in the present study contain around 1.7% nicotine in a liquid that is colorless to light yellow and has a pH of 8–9. The mild tooth discoloration observed in this group is likely attributable to the color of nicotine or the color of the tobacco flavoring ingredients added.

Red wine, coffee, and soy sauce are widely consumed worldwide and known to cause tooth discoloration [36,37]. Under similar conditions and the same duration of exposure, red wine caused more severe discoloration than CS, while coffee and soy sauce caused less severe discoloration. Discoloration of enamel was removable by 35% H_2O_2 in the CS and red wine group, while color mismatch between dental enamel and composite restorations persisted. In contrast, discoloration of enamel in both the coffee and soy sauce groups was completely reversed by 6% H_2O_2, and discolorations of dentin and composite restorations were eliminated by 35% H_2O_2. Increased discoloration in the red wine group is likely related to its low pH (3.36) relative to that of coffee (5.38) and soy sauce (4.13). A low pH may affect the surface integrity of dental hard tissues and enable easier penetration of pigments into the tissue matrices, leading to internalized discoloration that cannot be removed by mechanical means [38]. The findings of the present study conform to this rationale, as the degree of discoloration increased with descending pH from coffee to soy sauce to red wine.

Wide variations exist in discolorations of enamel, dentin, and composite resin restorations exposed to coffee, soy sauce, and red wine for the same length of time under similar conditions, suggesting that the underlying mechanisms may differ and are dependent on the types of colorants and substrates. Apart from the effect of pH discussed above, the organic and inorganic composition of the substrates may also be involved. While dental enamel is composed of 96% dense hydroxyapatite crystals with little organic content or water, dentin and composite resins contain 30%–50% organic polymers and more water, which may potentially absorb and react with pigments. In general, we found more discoloration in dentin and composite resin than in enamel, and whitening treatments were more effective for enamel.

5. Conclusions

In summary, our findings indicate that regular tooth brushing affects the outcomes of exposure on enamel, dentin, and composite resin restorations. It is therefore important to add tooth brushing to the study design of tooth discoloration studies *in vitro*. Common sources of colorants, such as the 4 we studied (cigarette smoke, red wine, soy sauce, coffee), cause differential discoloration in dental enamel, dentin, and composite resin restorations. Red wine and CS are more likely to cause color mismatch between enamel and composite resin restorations that cannot be corrected by whitening treatments. Although minor color changes caused by EC aerosol could be eliminated by brushing with a tooth-whitening toothpaste alone, whitening with 6% and 35% H_2O_2 was necessary to reverse discoloration caused by coffee and soy sauce.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.jdent.2019.103182.

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