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Combined effect of staining substances on the discoloration of esthetic Class V dental restorative materials

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Abstract The purpose of this study was to determine the combined effect of an organic substance (mucin as a substitute for salivary organic substances), chlorhexidine, and an iron compound/tea solution on the changes in the color of esthetic Class V dental restorative materials. Color of a glass ionomer, resin-modified glass ionomer, compomer and flowable resin composite of A2 shade, respectively, was determined according to the CIELAB color scale relative to the standard illuminant D65. Color was measured at baseline, and after sequential immersion in the following substances: Step-1, mucin in PBS (MCP) for 48 h; Step-2, chlorhexidine (CHX) for 24 h; Step-3, iron compound (IRN) or tea solution (TEA) up to 7 days; and Step-4, ultrasonic cleaning for 1 h. Color change (ΔE^*_{ab}) was calculated by the equation: $\Delta E^*_{ab} = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$, of which ΔL^* indicates changes in value, Δa^* indicates changes in red-green parameter and Δb^* indicates changes in yellow-blue parameter. ΔE^*_{ab} values after immersion in MCP and CHX were compared, and ΔE^*_{ab} values after immersion in IRN or TEA, and subsequent ultrasonic cleaning were compared with respect to the restorative material and immersion substance. ΔE^*_{ab} and changes in the color parameters (ΔL^*, ΔC^*_{ab} and ΔH^*_{ab}) were analyzed by repeated measures, analysis of variance and a post-hoc test at the 0.05 level of significance. Color changes after immersion in MCP were acceptable ($\Delta E^*_{ab} < 3.3$), and those after immersion in CHX were generally acceptable. The range of ΔE^*_{ab} values after immersion in IRN was 3.1–19.6, and that after ultrasonic cleaning was 2.4–9.6. The range of ΔE^*_{ab} values after immersion in TEA was 10.7–21.1, and that after ultrasonic cleaning was 11.9–14.5. Color changes of four Class V restorative materials after combined treatment with mucin, chlorhexidine and an iron compound/tea solution were not acceptable. Colors did not recover to their original values after ultrasonic cleaning. Modifications on the surface of a restoration should be considered to reduce stain accumulation.

1 Introduction

Plaque accumulation on teeth is generally obvious in poor oral hygiene areas such as cervical and proximal areas [1, 2], and the dental pellicle and plaque interact with staining substances. Brown discolorations appear most frequently on surfaces that favor the development of a thick pellicle [1]; therefore, cervical areas of teeth seem to be prone to discoloration. Staining was formed in the gingival third and interproximal areas of teeth by chlorhexidine [3].

Discoloration of teeth is classified as extrinsic, intrinsic or internalized, which may detract from esthetic appearance [2]. Restorative materials were regarded as artificial defects that promoted internalized discoloration [4, 5]. Extrinsic discoloration of teeth may be caused by retention of colored substances in plaque and/or acquired pellicle, or by chemical alteration of these organic integuments. It was found that 31% of men and 21% of women had moderate or severe extrinsic discoloration [1]. The rate of accumulation of deposits on dental acrylic resins was affected by saliva composition, dietary intake and the surface characteristics of a material [6], and dietary factors influenced tooth staining associated

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with the use of chlorhexidine [7]. Extrinsic discoloration of teeth following a large consumption of tannin-containing beverages or a prolonged use of chlorhexidine is well-known [8].

Although chlorhexidine, tannic acid and iron did not cause discoloration of acrylic resins when applied individually, exposure of dental plaque to either chlorhexidine or tannic acid before the application of iron produced marked staining [9, 10]. *In vitro* staining models for simulating intraoral staining have typically been evaluated by measuring the changes in optical density of acrylic resins [11–13]. However, color changes produced by simulated intraoral staining procedures may be quantified with a color-measuring spectrophotometer, which can provide the direction of changes in color parameters such as hue, chroma and value.

Since the cervical areas of teeth are prone to accumulation of the organic substances in the oral cavity and these organic substances can be altered by chemical substances, evaluation of possible staining on esthetic cervical restorative materials has clinical implications. However, there have been few studies on the staining of cervical restoratives under simulated oral conditions. The hypothesis of the present study was that there were acceptable changes in the color of esthetic Class V restorative materials, after combined immersion treatments with substances normally found in the oral cavity, regardless of the type of materials and immersion solutions. It was reported that 50% of observers considered unacceptable when ΔE^*_{ab} value was approximately 3.3 [14]; therefore, ΔE^*_{ab} value of 3.3 was considered as an acceptable limit.

The purpose of this study was to determine the combined effect of an organic substance (mucin as a substitute for salivary organic substance), chlorhexidine, and an iron compound/tea solution, on the changes in color and color parameters of esthetic dental materials used for the restoration of Class V cavity.

2 Material and methods

Typical esthetic materials used for the restoration of cervical areas were selected, namely, one glass ionomer (KFP), one resin-modified glass ionomer (PFQ), one compomer (F20) and one flowable resin composite (FSF) of respective A2 shade (Table 1).

Specimens of 10 mm in diameter and 2 mm in thickness were made with a polytetrafluoroethylene mold. Ten specimens were made for each material. Specimens were immersed in distilled water at 37°C for 24 h before baseline color measurement. Color was measured, according to the CIELAB color scale relative to the standard illuminant D65 over a white standard tile, on a reflection spectrophotometer (Color-Eye 7000, GretagMecbeth Instruments Corp., New Windsor, NY, USA) after blot drying. An ultraviolet (UV) component of illumination was included, and the spectral component was excluded (SCE mode) [15]. The aperture size was 3 × 8 mm, and illuminating and viewing configuration were CIE diffuse/8° geometry. Measurements were repeated three times for each specimen.

Simulated staining procedures consisted of four steps. Immersion solutions are listed in Table 2. Amounts of immersion solutions were 15 ml/specimen.

Step-1 was forming an initial pellicle-like layer with mucin. Specimens were immersed in MCP at 37°C for 48 h, and their color was measured at 24 and 48 h.

Step-2 was chemical alteration of adsorbed mucin with chlorhexidine [21]. Specimens were immersed in 37°C CHX for 24 h.

Step-3 was the determination of the influence of an iron compound or a tea solution on staining. Of ten specimens, five specimens were allocated to each of the two experimental groups of IRN and TEA in this step. Specimens were immersed in IRN or TEA up to 7 days, and color was measured at 24 h, 48 h and 7 days. After Step1, 2 and 3, the color

Table 1 Materials used in this study

Species	Code	Brand name	Batch number	Manufacturer
Glass Ionomer	KFP	Ketac-Fil Plus Aplicap	172469	3M ESPE, St. Paul, MN, USA
Resin-modified glass ionomer	PFQ	Photac-Fil Quick Aplicap	172360	3M ESPE
Compomer	F20	F2000	4EX	3M ESPE
Flowable resin composite	FSF	Filtek Supreme Flowable	4EC	3M ESPE

Table 2 Immersion solutions

Code	Immersion solution	Concentration	Batch number	Manufacturer
PBS	Phosphate buffered saline	0.01 M	014K8210	Sigma, St. Louis, MO, USA
MCP	Mucin in PBS	3 g/l in PBS [6, 16]	113K1012	Sigma
CHX	Chlorhexidine digluconate	0.2% in PBS [17, 18]	307–826	STERIS Co., St. Louis, MO, USA
IRN	Ammonium iron (III) citrate	10 mM in DW (pH 7.1) [19]	043K0017	Sigma
TEA	Tea solution	1 g/100 ml in DW [18, 20]	3K25SB052	Lipton, Englewood Cliffs, NJ, USA

of the specimens was measured after drying for 2 h in an oven at 37°C instead of blot drying because deposits could be removed during blotting.

Step-4 was removal of deposits from the specimen. Specimens were ultrasonically cleaned for 1 h, and color was measured after blot drying.

Color difference was calculated based on the CIELAB color scale as $\Delta E^*_{ab} = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$, of which ΔL^* indicates changes in value (lightness), Δa^* indicates changes in red-green parameter and Δb^* indicates changes in yellow-blue parameter. Changes in value (ΔL^*), chroma (ΔC^*_{ab}) and hue (ΔH^*_{ab}), after immersion in IRN or TEA for 48 h, were determined as a function of the material. ΔL^* was calculated as 'CIE L^* value after immersion in IRN/TEA for 48 h – CIE L^* value at the baseline', change in chroma was calculated as $\Delta C^*_{ab} = (\Delta a^{*2} + \Delta b^{*2})^{1/2}$, and change in hue was calculated as $\Delta H^*_{ab} = [(\Delta E^*_{ab})^2 - (\Delta L^*)^2 - (\Delta C^*_{ab})^2]^{1/2}$ [15]. Hue describes the dominant color of an object, value intensifies the lightness or darkness of a color, and chroma represents the degree of saturation of a particular hue.

Repeated measures, analysis of variance (ANOVA) was used to compare differences in changes of color with the independent variables of the type of material and the immersion solution (SPSS 11.0, SPSS, Chicago, IL, USA, $p = 0.05$). Means were compared with Scheffe's multiple comparison test at the 0.05 level of significance [22].

3 Results

Color changes after immersion in MCP (Step1) and CHX (Step2) from the baseline are shown in Fig. 1. The range of ΔE^*_{ab} after immersion in MCP was 0.5–2.0, and that in CHX was 1.6–5.3. Color changes of a resin-modified glass ionomer (PFQ) were higher than those of other materials.

ΔE^*_{ab} values after immersion in IRN are shown in Fig. 2. The range of ΔE^*_{ab} after immersion for 24 h was 4.5–11.0, that for 48 h was 13.6–19.6, that for 7 days was 3.1–11.7, and that after ultrasonic cleaning (US Clean) was 2.4–9.6. In all four conditions, a glass ionomer (KPF) showed the highest color changes, whereas PFQ showed the lowest. Color changes after 48 h immersion were the highest in all four materials.

ΔE^*_{ab} values after immersion in TEA are shown in Fig. 3. The range of ΔE^*_{ab} after immersion for 24 h was 10.7–14.7, that for 48 h was 17.9–21.1, that for 7 days was 16.7–20.8, and that after ultrasonic cleaning was 11.9–14.5. Color change after 48 h immersion was the highest. Color changes after immersion in TEA were higher than those after immersion in IRN, and they were also significantly different after ultrasonic cleaning ($p < 0.05$).

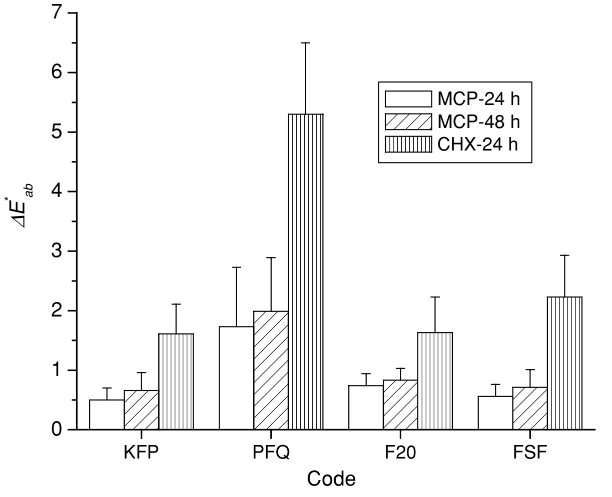

Fig. 1 Color changes after immersion in mucin and chlorhexidine

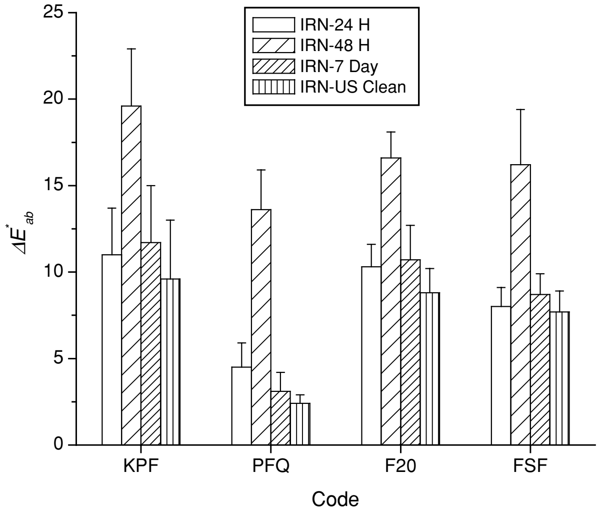

Fig. 2 Color changes after immersion in IRN

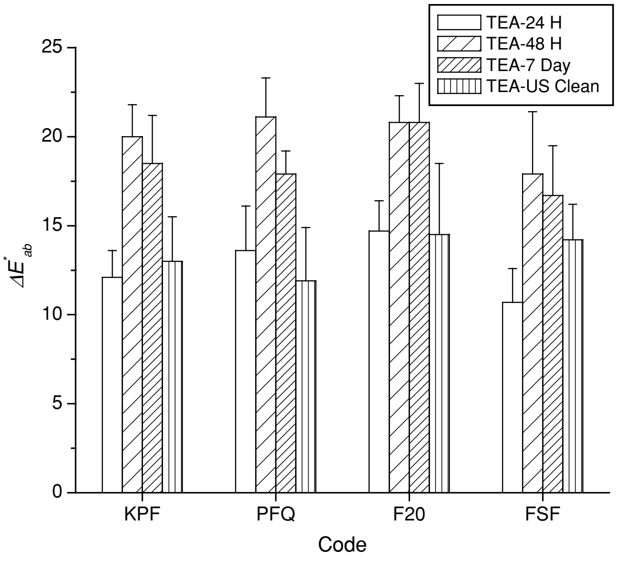

Fig. 3 Color changes after immersion in TEA

Table 3 ΔL^*, ΔC^*_{ab} and ΔH^*_{ab} values after immersion in IRN for 48 h

Code	ΔL^{*}[a]	ΔC^*_{ab}	ΔH^*_{ab}
KPF (1)[b]	−3.9 (1.9)[c]	11.3 (5.0)	16.7 (1.6)
PFQ (2)	−0.2 (1.9)	5.5 (2.5)	12.9 (1.8)
F20 (3)	−2.2 (0.7)	9.4 (2.0)	13.3 (1.1)
FSF (4)	−1.6 (1.1)	8.9 (3.2)	13.6 (1.7)
DG	1<2	2<1	2.3.4<1

[a] ΔL^*, ΔC^*_{ab} and ΔH^*_{ab} mean the changes in CIE L^*, chroma and hue from the baseline

[b] These numeric codes are used in DG. DG = Different groups by the specimen condition. "<" means significantly different group marker (Scheffe test, $p < 0.05$)

[c] Standard deviations are in parentheses

Table 4 ΔL^*, ΔC^*_{ab} and ΔH^*_{ab} values after immersion in TEA for 48 h

Code	ΔL^{*}[a]	ΔC^*_{ab}	ΔH^*_{ab}
KPF (1)[b]	−11.5 (1.7)[c]	6.7 (1.3)	14.9 (0.8)
PFQ (2)	−14.9 (2.0)	5.9 (0.9)	14.3 (0.5)
F20 (3)	−12.4 (1.5)	10.8 (2.1)	14.3 (1.1)
FSF (4)	−9.3 (1.6)	8.9 (1.7)	13.0 (1.3)
DG	2<3.1<4	2.1<4<3	4<2.3.1

[a] ΔL^*, ΔC^*_{ab} and ΔH^*_{ab} mean the changes in CIE L*, chroma and hue from the baseline

[b] These numeric codes are used in DG. DG = Different groups by the specimen condition. "<" means significantly different group marker (Scheffe test, $p < 0.05$)

[c] Standard deviations are in parentheses

Color changes (ΔE^*_{ab}) after combined immersion in mucin (48 h), chlorhexidine (24 h), and an iron compound/tea solution (24 h, 48 h and 7 days) were influenced by the type of restorative material, the immersion solution and the evaluation period after immersion in an iron compound/tea solution ($p < 0.05$).

Changes in color parameters (ΔL, ΔC^*_{ab} and ΔH^*_{ab}) after immersion in IRN for 48 h from the baseline are listed in Table 3. Lightness decreased after immersion treatment. The change in KPF was higher than that in PFQ, and ΔL^* values generally showed high standard deviations. Changes in chroma and hue (ΔC^*_{ab} and ΔH^*_{ab}) in KPF were higher than the other three materials.

Changes in color parameters after immersion in TEA for 48 h from the baseline are listed in Table 4. Lightness decreased after immersion treatment. The change of CIE L^* value in PFQ was higher than those of the other three materials. ΔH^*_{ab} of FSF was lower than the other three materials ($p < 0.05$). Changes in CIE L^* values after immersion in TEA were significantly higher than those after immersion in IRN.

4 Discussion

The hypothesis of the present study was rejected because the changes in color were not acceptable after the combined treatment with mucin (a substitute for salivary organic substances), chlorhexidine and an iron/tea solution ($\Delta E^*_{ab} > 3.3$) except a few cases [14], and the changes in color were influenced by the type of material and immersion solution. However, color changes after immersion in MCP for 48 h were acceptable, and those after immersion in CHX for 24 h were acceptable except for PFQ (Fig. 1). High standard deviations for color changes after immersion in CHX (1.6 ± 0.5 for KFP, 5.3 ± 1.2 for PFQ, 1.6 ± 0.6 for F20 and 2.2 ± 0.7 for FSF) seems to reflect the fact that white powder-like deposits made on the surface of the specimens as a result of immersion in mucin were not firmly attached. The amount of deposition of chemically altered MCP by CHX may influence the amount of staining (discoloration) after subsequent IRN or TEA treatment. If it were assumed that color change after CHX treatment reflected the amount of MCP depositions (higher color change indicates more deposits), the amount of deposits on PFQ seemed to be the highest. After CHX treatment, color changes of PFQ was the highest; however, color changes after immersion in IRN for 24 and 48 h were lowest in PFQ. Therefore, the influence of the amount of altered organic deposits on the stain accumulation by IRN was not obvious from the present study. In case of TEA treatment, there was no significant correlation between ΔE^*_{ab} values after immersion in CHX and TEA based on regression analysis ($p > 0.05$). The mean thickness of the specimens of the present study was 2 mm, which is less than infinite optical thickness (true color of a translucent material regardless of the thickness) for dental resin composite [23]. Instrumental color measurement of translucent materials is influenced by the properties of background. However, regardless of the type of background used for color measurement, differences in colors will be consistent when color is measured over the same background [24].

looseness-1 ΔE^*_{ab} values for four materials after immersion in IRN up to 7 days, and those after ultrasonic cleaning were not acceptable (Fig. 2). Since ΔE^*_{ab} values after 48 h were the highest, changes in color parameters were analyzed at this point (Table 3). Standard deviations were high in ΔL^* values, which might reflect the fact that the amount of accumulated staining varied by the specimens and by the area of the specimens, although they were prepared and treated in the same way. Changes in hue (ΔH^*_{ab}) were as high as 12.9–16.7. However, the practical meaning of changes in hue is still unclear. Although the practical implications of ultrasonic cleaning procedure should be further studied, this step was performed to remove loosely attached stains. After ultrasonic cleaning, color changes in three of the four materials were still perceptible ($\Delta E^*_{ab} > 3.3$), which indicates that extrinsic as well as internalized discoloration occurred [5]. ΔE^*_{ab} values for the four materials after immersion in TEA up to 7 days, and those after ultrasonic cleaning were not acceptable (Fig. 3). In the present study, stain removal was performed by ultrasonic stain removal for simple comparison; however, if bleaching agents were used for this step, the results might have been different.

In dental field, many of the studies for the influence of organic substances on the stain accumulation were focused on the denture base materials. Therefore, the protocols used for the acrylic resins were adapted in the present study. Although the surface properties of methacrylate and filled dimethacrylate can not be the same, basic properties would be similar [25]. Dental acrylic resin specimens were treated with saliva to form an initial pellicle layer and facilitate the uptake of the stain [12]. Organic layers decreased the surface free energy of dental acrylic resins and imparted a more basic character [26]. Cationic antiseptics, such as chlorhexidine, can precipitate or bind to surface anionic chromogens contained in foods and beverages [17, 18]. The major mechanism of chlorhexidine to induce dental staining together with ferric ions has been reported as denaturation, and iron sulfide might be one important cause for extrinsic dental stain [19]. Pretreatment with chlorhexidine or tannic acid led to marked discoloration of the integument on the tooth followed by iron application in a human model [8], and the addition of chlorhexidine and saliva increased staining of indirect resin composites when used with tea [20]. Based on the above references for tooth and acrylic resin, staining protocols in the present study were developed. As the results, iron and tea interacted with mucin and chlorhexidine treated restorative materials, which resulted in very high color changes ($\Delta E^*_{ab} = 4.5$–19.6 in an iron solution and 10.7–21.1 in a tea solution). Although there have been few reports on the clinical staining of esthetic restorations, this staining protocol may be used to determine the stain susceptibility of restorative materials. Analysis on the composition of stain may provide more insight on the mechanism of staining. Further studies are recommended.

The tea used in the present study made high and consistent stain on all four materials. The adsorption of black tea onto a pellicle-like layer formed on hydroxyapatite discs has been reported [27]. Black tea components have been shown to have a profound effect on *in vitro* pellicle maturation, which were not eluted by either phosphate buffer or sodium dodecyl sulphate rinses [27]. Therefore, ultrasonic cleaning was tried in the present study; however, it could not remove the staining either. This result suggests that the internalized discoloration of resin composites occurred.

Staining materials are divided into two categories: those which produce a stain as a result of their basic color, and those which cause staining by chemical interaction [5]. Iron compound and tea (tannin-containing beverage) tested in the present study might have acted in both ways because both of them are denaturing agents [8], and their colors are similar to that of the stain.

The amount of plaque deposition on tooth and prosthetic materials seemed to be related to the degree of their surface roughness, while plaque formation was qualitatively similar [28]. In the present study, the surface characteristics of the four materials should be different, although the surface roughness was similar because a Mylar strip was applied before curing and no polishing was done. Color changes were not acceptable after immersion in iron compound and tea solutions, and varied with the type of material, which reflects different surface properties. Plaque accumulation was reduced and plaque removal facilitated by the tetrafluoroethylene on dental alloys [29]. This method can be applied on polymer-based restorative materials to reduce plaque accumulation and subsequent staining by chlorhexidine and dietary factors.

Within the limitations of this study, the following conclusions were drawn:

1. Color changes, after combined treatments with mucin, chlorhexidine and an iron compound/tea solution were not acceptable except a few cases.
2. After combined treatment, value (lightness) decreased and chroma increased. Changes in hue were very high.
3. After immersion in iron compound, resin-modified glass ionomer showed the least color change, but all materials showed similar and high color changes in tea solution.

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