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Staining of Hybrid Composites with Coffee, Oolong Tea, or Red Wine

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This study examined the surface staining mechanism of a photopolymerized composite by coffee, oolong tea, and red wine.

Dental composite was subjected to an experimental 24-hour staining cycle: 17-hour immersion in artificial saliva solution containing 0.3% mucin followed by 7-hour immersion in coffee, tea, or wine. After one, two, and four weeks, digital images of the composite surface were analyzed in grayscale mode with an imaging analyzer. Specimens polished but not immersed were used as a baseline measurement for color change. Additionally, the effects of mechanical brushing and chlorhexidine on drink-induced staining were examined.

Wine caused the most severe staining, followed by tea and coffee. After four weeks of immersion, brushing reduced surface staining by wine. On the contrary, chlorhexidine increased the staining effect of tea and coffee ($p < 0.05$) when compared to the control specimens. In conclusion, we showed that common drinks stained the dental composite, but each by a specific mechanism that depended on external conditions such as the presence of chlorhexidine.

Key words: Light-cured resin composite, Staining, Chlorhexidine

INTRODUCTION

Resin composites are widely used because they have excellent esthetic properties and can be bonded to dentin or enamel. Their increasing popularity can be attributed to demands from patients for tooth-colored restorations. Historically, one common reason for the replacement of chemical-cured resins was an unacceptable color match with teeth¹. Color changes in resins occur from intrinsic and extrinsic factors. Historically, one major intrinsic factor causing long-term discoloration of resins has been the oxidation of monomers or catalysts. However, light-cured formulations have dramatically reduced intrinsically-mediated discoloration because benzoyl peroxide is excluded from these systems^{2,3}. Extrinsic factors such as adsorption or absorption of extrinsic stains, on the other hand, still pose a major problem for esthetic restorations. Vogel reported that extrinsic stains result from discoloration by pellicle bacterial plaque⁴. Eriksen and Nordbø⁵ suggested that there are at least three mechanisms that contribute to the formation of extrinsic stains: (a) production of colored components in plaque by chromogenic bacteria; (b) retention of colored substances from dietary constituents passing through the oral cavity; and (c) formation of colored products from the chemical transformation of pellicle components.

Studies measuring the accumulation of pellicle in

humans suggest that factors such as diet (coffee, tea, and red wine), smoking, exposure to antimicrobial agents⁶, and daily teeth cleaning affect extrinsic stain development⁷⁻⁹. Drinks such as coffee, tea, and wine are commonly consumed between meals, and brushing frequently thereby exposes the composites. Extrinsic dental stains caused by cationic antiseptics, such as chlorhexidine (CHX), are also a well observed and studied phenomenon^{10,11}. Mechanism of CHX-induced staining appears to be caused by the binding of cationic antiseptics to the dietary chromogens¹².

Our present study focused on comparing the staining effects of three drinks - coffee, oolong tea, and red wine - on a contemporary composite in artificial saliva. In addition, the ability of brushing to prevent staining was investigated, as well as the combined influence of both staining drinks and CHX. Staining from oolong tea has not been previously reported, and likewise for the potential synergism between artificial saliva, extrinsic staining drinks, brushing, and CHX. The hypothesis of this study was that staining of composites was time-mediated, and that it depended on the salivary components as well as the interaction between these factors and the nature of the drink itself.

Table 1 Products used for staining effect

Product	Manufacturer	Trade name	Ingredients
Coffee	UCC, Kobe, Japan	Black muto	Coffee without sugar and milk
Oolong tea	Asahi, Tokyo, Japan	Asahi oolong tea	Oolong tea
Red wine	CGC, Tokyo, Japan	Collericcio rosso	Less than 11.5% alcohol

MATERIALS AND METHODS

A hybrid resin composite (Clearfil AP-X A2, Kuraray Medical Inc. Co., Tokyo, Japan) was used to make disk specimens 10 mm in diameter and 5 mm thick. The free surface of the material was covered with a transparent matrix (Mylar[®], 0.1 mm thick) and photocured three times for 40 seconds each time to ensure maximal polymerization using a photocuring unit (Optilux 401, Demetron Kerr, CA, USA, 500 mW/cm²). Staining measurement was done only on this free surface. After polymerization, specimens (n=3 per condition) were wet-polished with SiC papers (800, 1000, 1500, and 2000 grit, successively). The 2000-grit surface was deemed equivalent to a highly polished clinical surface.

Three types of drink were tested in the present study based on their common consumption in Japan: coffee, oolong tea, and red wine (Table 1). Staining of the composite specimens was performed using the following paradigm. Specimens were first placed in double-distilled water or artificial saliva containing 0.3% mucin (Table 2) for 17 hours. After washing with water, they were immersed into one of the drinks for seven hours. The artificial saliva composition was selected based on the major organic constituents of human saliva (Table 3) and the inorganic components of commercially available artificial saliva (Table 4). Control specimens were transferred to water or artificial saliva instead of the drink solution. These 24-hour protocols were repeated daily for one, two, and four weeks. Other controls (to serve as baseline measurement) were not immersed in any solution.

Brushing without toothpaste was used post-exposure to the drinks to determine its mechanical effect on staining. At the end of the 7-hour exposure, specimens were brushed for 10 seconds using a commercial electrical-powered toothbrush (GUM, Model TS-40S, Sunstar Inc., Osaka, Japan) with a 200 g load. Brushing was repeated at the end of each 24-hour immersion cycle followed by washing with water. This procedure was conducted using a custom-made device to fix the specimens in the same place while controlling the load.

To test the chemical effect of anti-plaque agents on staining by drinks, specimens were immersed in chlorhexidine (0.2% v/v from 5% hibitane concentrate, Sumitomo Pharmaceuticals Co. Ltd., Osaka,

Table 2 Components of artificial saliva

KCl	1.20 g
NaCl	0.84 g
CaCl ₂	0.15 g
MgCl ₂	0.05 g
K ₂ HPO ₄	0.34 g
Mucin	3.00 g (0.3%)
Distilled water	1,000 ml

Table 3 Components of human saliva

Inorganic matter	0.23%
K	0.06%
HCO ₃	0.1 %
Cl	0.05%
Na	0.02%
Ca	0.01%
SCN	0.03%
P	0.02%
Organic matter	0.5%
Mucin	0.3%
Ptyalin	Particle
Urea	Particle
Water	99.27%

Table 4 Components of artificial saliva, Saliveht (Teijin, Tokyo, Japan)

KCl	60.0 mg
NaCl	42.2 mg
K ₂ HPO ₄	17.1 mg
CaCl ₂	7.3 mg
MgCl ₂	2.6 mg < per 50g >

Japan) for 10 minutes after exposure to the drinks in each 24-hour cycle, followed by washing with water.

At the end of the staining procedure, all specimens were washed with water. They were then dried and digitally photographed (Camedia C-2000 Zoom, Olympus, Japan) under standardized conditions (light: 500 W × 4, distance: 10 cm, zoom: ×2). The most popular method to measure color difference is the L*a*b* method. However, we used grayscale analysis (rather than the L*a*b* method) to assess

the degree of staining because it allowed us to directly and fairly compare staining by chromogens with diverse hues and chromas. Photographs were analyzed in grayscale mode using a standard image analysis software (1D Image Analysis, Kodak Digital Science, Japan). Black vinyl tape was used for calibration (grayscale=256). The grayscale values of specimens in each treatment group were then averaged. Due to interactions between factor level means, two-way ANOVA was not valid. Therefore the data were compared using serial one-way ANOVA and Tukey's post-hoc analysis ($\alpha = 0.05$).

RESULTS

Fig. 1 shows the pictures of representative specimens at four weeks.

When stored in water, coffee, tea, and wine caused no significant changes in the grayscale value of the resin composite as compared to those treated with water or those not immersed in any solution (Fig. 2). However, when artificial saliva with mucin was used, grayscale values increased significantly after one week of exposure, regardless of whether coffee, tea, wine, or nothing was added to the treatment cycle (Fig. 3). It can be seen from Fig. 3 that

after one week, coffee and tea stained the composite less (by 10-15 grayscale units) than wine and saliva with mucin. Beyond one week, the coffee specimens remained unchanged, the tea specimens stained 10-15% more in the second week than in the first week, but wine caused significant increase in staining. By itself, saliva with mucin did not cause any increase in staining after one week.

The effects of brushing and CHX are shown in Figs. 4 and 5 respectively, and their effects are directly compared in Fig. 6. Brushing the specimens at the beginning of each treatment cycle reduced but did not eliminate the staining caused by mucin alone (Figs. 3, 4, 6) and did not prevent staining by coffee, tea, or wine. Brushing also changed the staining patterns of the drinks (Fig. 4). Staining by tea, in particular, seemed resistant to removal by brushing, whereas wine staining was reduced by brushing.

The addition of chlorhexidine (CHX) to the treatment cycle markedly reduced the staining by mucin alone (Figs. 3, 5, 6), but markedly increased the staining of all drink solutions. CHX modestly increased staining in saliva, but augmented staining by coffee, tea, and wine - particularly at the longer treatment intervals of two weeks and four weeks.

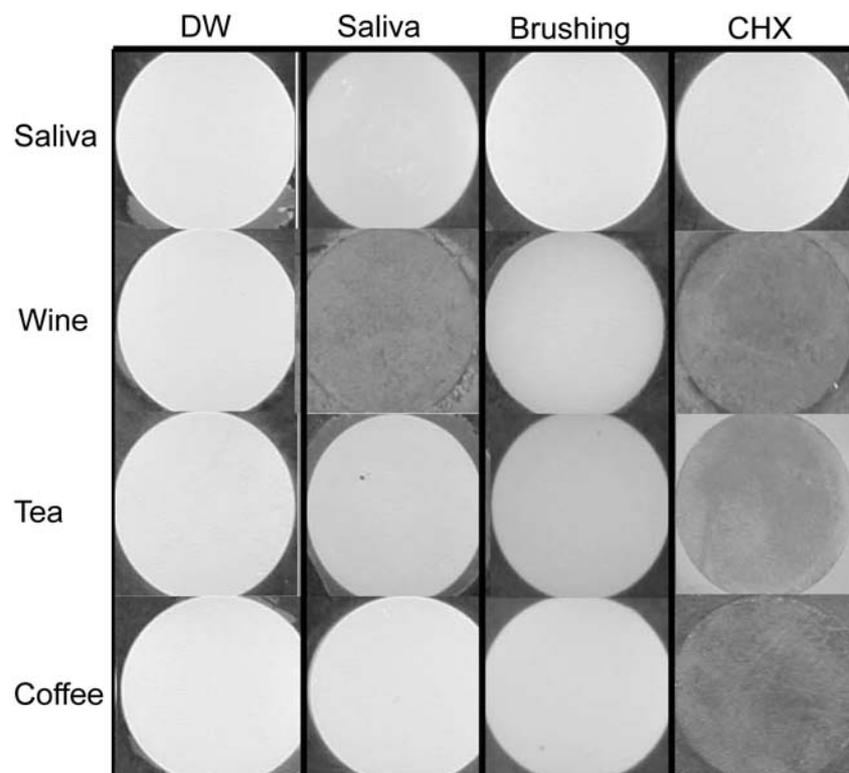


Fig. 1 Pictures of representative specimens at 4 weeks.

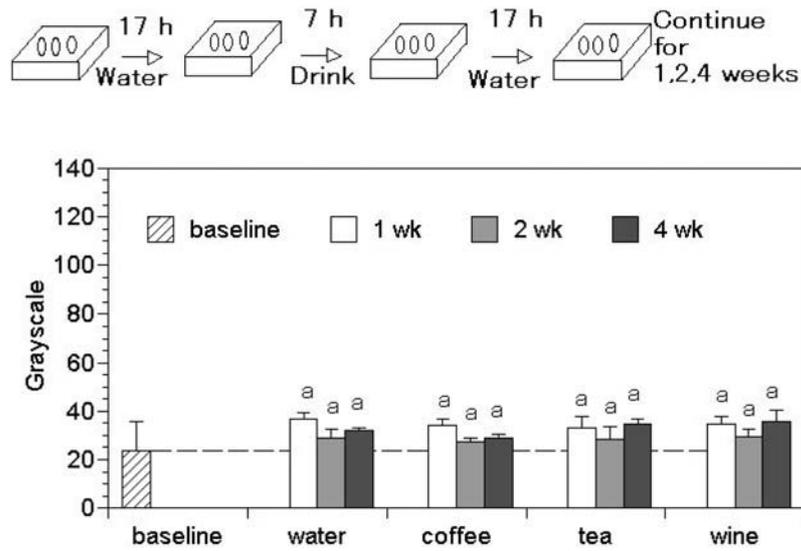


Fig. 2 Staining of composites (grayscale value) after immersion in distilled water, coffee, oolong tea, or red wine for 1-4 weeks. Specimens were stored in distilled water for 17 h, then immersed in one of the drinks or water (control) for 7 h. Error bars indicate standard deviations ($n=3$). Letters indicate statistical comparisons among time periods (one-way ANOVA and Tukey's multiple comparison test, $\alpha=0.05$). Same letter indicates no significant differences. Dashed line indicates control baseline (no immersion).

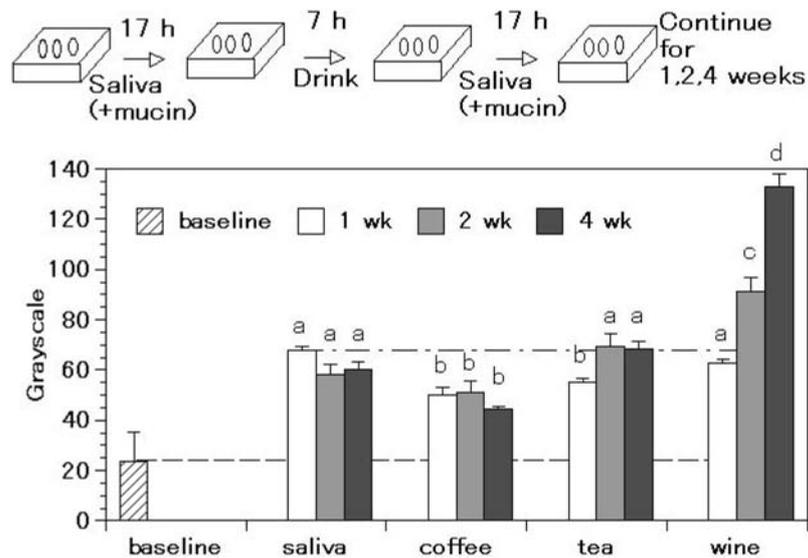


Fig. 3 Staining of composites (grayscale value) after immersion in artificial saliva, coffee, oolong tea, or red wine for 1-4 weeks. Specimens were stored in artificial saliva containing 0.3% mucin for 17 h, followed by immersion in each of the drinks or saliva for 7 h. Error bars indicate standard deviations ($n=3$). Letters indicate statistical comparisons among time periods (one-way ANOVA and Tukey's multiple comparison test, $\alpha=0.05$). Same letter indicates no significant differences. Lower dashed line indicates control baseline (no immersion), while upper dashed line indicates controls exposed to saliva but not drinks.

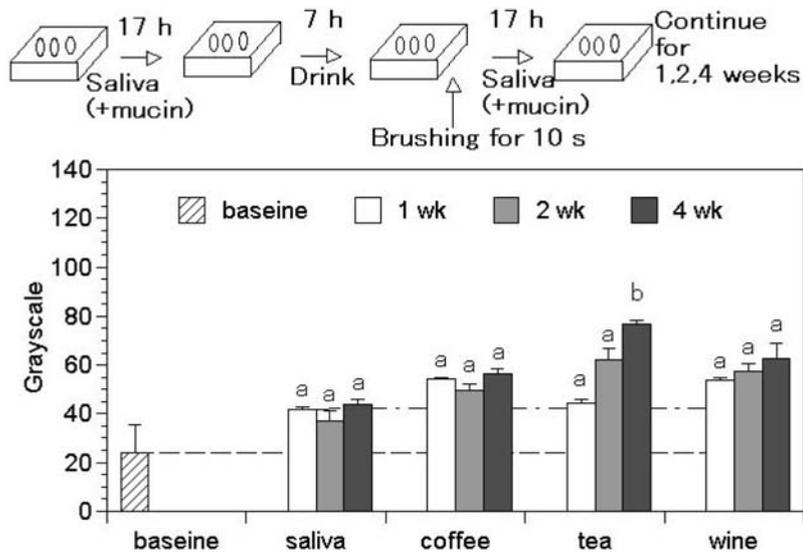


Fig. 4 Composite stain after toothbrushing. Specimens were stored in artificial saliva containing 0.3% mucin for 17 h, followed by immersion in each of the three drinks for 7 h. They were then brushed with an electrical-powered toothbrush with a 200 g load for 10 s. Error bars indicate standard deviations ($n=3$). Letters indicate statistical comparisons among time periods (one-way ANOVA and Tukey's multiple comparison test, $\alpha=0.05$). Same letter indicates no significant differences. Lower dashed line indicates control baseline (no immersion), while upper dashed line indicates controls exposed to saliva but not drinks.

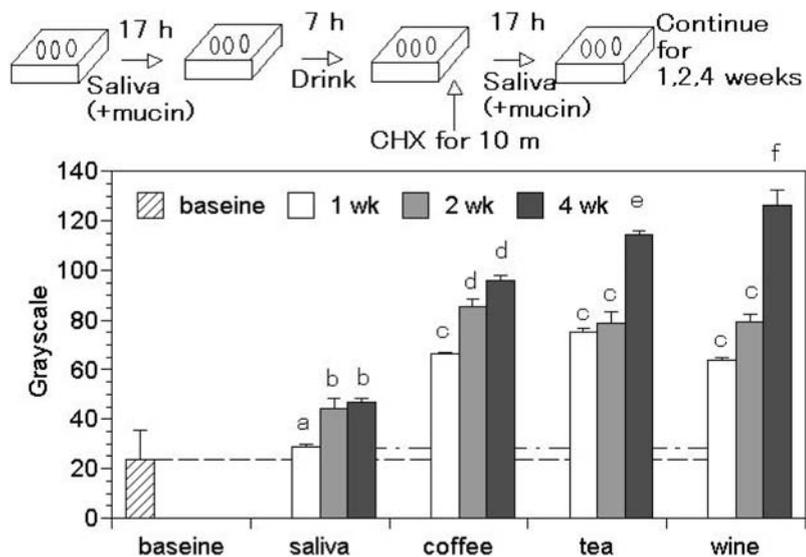


Fig. 5 Composite staining from drinks and chlorhexidine (CHX). Specimens were stored in artificial saliva containing 0.3% mucin for 17 h, then in each of the three drinks for 7 h, and finally immersed in 0.2% CHX solution for 10 min. Error bars indicate standard deviations ($n=3$). Letters indicate statistical comparisons among time periods (one-way ANOVA and Tukey's multiple comparison test, $\alpha=0.05$). Same letter indicates no significant differences. Lower dashed line indicates control baseline (no immersion), while upper dashed line indicates positive controls exposed to saliva but not drinks.

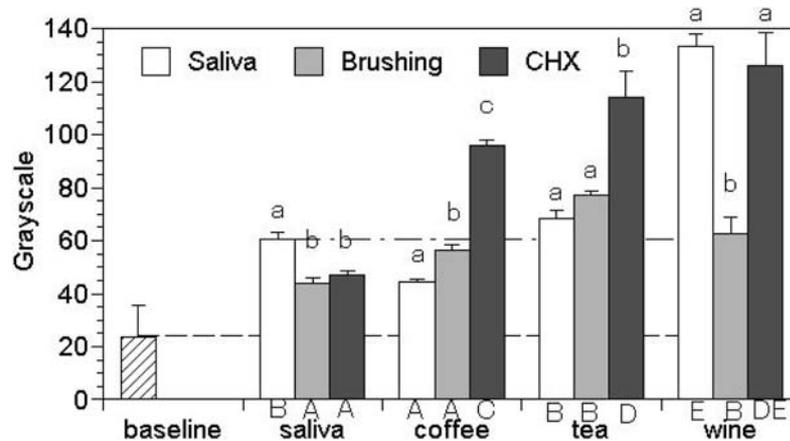


Fig. 6 Influence of saliva, after brushing and CHX, on composite staining from drinks at 4 weeks. Error bars indicate standard deviations ($n=3$). Lowercase letters indicate statistical comparisons among specimen conditions (one-way ANOVA and Tukey's multiple comparison test, $\alpha=0.05$). Uppercase letters indicate comparisons among all groups (one-way ANOVA and Tukey's multiple comparison test, $\alpha=0.05$). Same letter indicates no significant differences. Lower dashed line indicates control baseline (no immersion), while upper dashed line indicates positive controls exposed to saliva but not drinks.

DISCUSSION

When composite specimens were immersed in distilled water only, no staining was observed over four weeks (Fig. 2). Artificial saliva (with 0.3% mucin) by itself caused staining within one week, but staining did not increase with time (Fig. 3). We speculated that staining was most likely caused by mucin, which is yellowish in color. This speculation was based on the data in Fig. 3, where saliva with mucin - but not water - caused increased staining; hence, the most likely cause was mucin. These results agreed with a previous report which suggested that extrinsic staining of composites was mediated by saliva¹³. Brushing and CHX decreased saliva staining significantly (Figs. 4, 5, 6), implying that staining was a surface phenomenon that could be removed (by brushing) or prevented from forming (by CHX).

Although coffee and oolong tea did not cause severe staining as compared to wine (Fig. 3), staining by coffee was increased if the surface was brushed (Figs. 4, 6). This result suggested that saliva with mucin might have formed a surface barrier that limited staining by coffee, and that removal of this barrier by brushing led to increased staining. This tentative role of saliva with mucin needs further research to verify its barrier mechanism on the surface of composites. As for CHX, it increased the staining effect of coffee and tea (Fig. 5, 6). It could be that CHX had replaced mucin, thereby causing staining; or that CHX further cemented the previously formed

stains. Additional research is needed to verify the effect of these potential mechanisms.

Wine staining was saliva-dependent (Figs. 2, 3). Since brushing decreased staining significantly (Figs. 4, 6), mucin might have promoted staining by wine. It has been previously reported that alcohol tends to degrade the surface properties of resin composites¹⁴⁻¹⁶. As a result, a rougher and degraded surface provides increased surface area for the adsorption of pigments, thus leading to more staining^{15,16}. Therefore, it could be possible that the alcohol component in wine roughened the composite surface, thereby resulting in increased staining. Alternatively, it could be that some ingredients in wine were able to stain the layer of mucin on the resin composite surface. Indeed, further studies are needed to assess the interactive staining effects of composites, wine, and mucin.

Brushing results indicated that pellicle formed on resin composite surface could be mechanically removed by brushing¹⁷. With wine, brushing effectively suppressed the staining. It was thought that since the surface had been degraded by alcohol, staining could be more easily removed from the compromised surface by brushing. Nonetheless, the exact mechanism was not clear.

Coffee and tea stained the composite specimens as much as wine when used with CHX (Fig. 5). This could be due to a different mechanism of staining by CHX. There is no doubt that cationic antiseptics, such as CHX, can precipitate or bind to anionic

chromogens from foods and beverages on tooth and resin surfaces^{18,19}.

To assess the validity of grayscale analysis method in measuring color difference, pictures of four-week specimens were shown in progressive mode (Fig. 1). In conjunction, the actual color variations were presented in graphs. Upon comparison between Fig. 1 and the color variations in graphic forms, it can be seen that the method used in this study was indeed good enough to measure color change.

In conclusion, our study demonstrated the complex interactive effects between common drinks and salivary components that led to staining. These staining effects were altered by mechanically- or chemically-induced changes in saliva deposition, suggesting that complex mechanisms were in play during staining. We also observed distinctly different staining mechanisms for wine, tea, and coffee. Future studies need to be undertaken to unravel the mechanisms of staining, whereby investigations will require more complex but more informative methods to measure color change.

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