J Mater Sci: Mater Med (2007) 18:685–691
DOI 10.1007/s10856-006-0011-3

Combined effects of staining substances on resin composites before and after surface sealant application

Yong-Keun Lee · John M. Powers

Received: 13 September 2005 / Accepted: 8 December 2005 / Published online: 30 November 2006

Abstract The objective was to measure the combined effect of mucin, chlorhexidine and tea solution on the staining of four dental resin composites, and to determine the effect of surface sealant on staining. One side of cured resin composite specimens of 10 mm in diameter and 2 mm in thickness were polished with 600-grit silicon carbide paper. One group of specimens ($n = 5$) was treated with a surface sealant [BisCover, Bisco, USA; SS (surface sealant) group], and the other group was not (NO group; control). Specimens were sequentially immersed in the following substances: Mucin in phosphate buffered saline (PBS); chlorhexidine; tea solution; and ultrasonic cleaning and then immersion in PBS. Color was measured on a reflection spectrophotometer. Changes in color (ΔE^*_{ab}) and color parameters, such as hue, chroma and value, after immersion in tea solution and subsequent cleaning were analyzed by repeated measures, analysis of variance at the 0.05 level of significance. The range of ΔE^*_{ab} values after immersion in tea solution was 11.4–21.1 for NO group and 10.5–19.6 for SS group, and that after cleaning was 2.4–10.0 for NO group and 2.7–8.3 for SS group. After staining, CIE L^* value (lightness) decreased, and CIE a^* and b^* values increased. Color changes of resin composites were not acceptable after sequential immersion treatment ($\Delta E^*_{ab} > 3.3$). The changes in color and color parameters of sealant applied group were not significantly different from those of control group except for a few combinations of color parameters and resin composites.

1 Introduction

Discoloration of teeth is classified as extrinsic, intrinsic and internalized discoloration, which interferes with esthetics. Dental restorative materials are regarded as artificial defects that promoted internalized discoloration [1, 2]. Extrinsic discoloration of teeth following a large consumption of tannin-containing beverage or a prolonged use of chlorhexidine is well-known [3].

Although chlorhexidine, tannic acid or iron did not cause discoloration of dental resins when applied individually, exposure of dental plaque to either chlorhexidine or tannic acid before the application of iron compound produced marked staining [4, 5]. Organic layers decreased the surface free energy of dental acrylic resins [6], and acrylic resin specimens showed increased uptake of stain by forming an initial pellicle layer when a salivary pellicle layer was formed on the surface [7].

Saliva contains immunoglobulins, proteins, enzymes, mucins and nitrogenous products [8]. Mucins are the principal organic constituents of mucus, which coat all mucosal surfaces [9]. The first deposits to accumulate on dental resins in the oral cavity are usually mucin and food, and the subsequent accumulation of plaque on the

---

Y.-K. Lee (✉)
Department of Dental Biomaterials Science, Dental Research Institute, College of Dentistry, Seoul National University, 28 Yeongeon-dong, Jongro-gu, Seoul, Korea
e-mail: ykleedm@snu.ac.kr

J. M. Powers
Department of Restorative Dentistry and Biomaterials, Houston Biomaterials Research Center, The University of Texas Dental Branch, Houston, TX, USA

dental resins then acts as a matrix for the deposition of stain [10]. Cationic antiseptics, such as chlorhexidine, can precipitate or bind to surface anionic chromogens contained in foods and beverages [11, 12]. The addition of chlorhexidine and saliva increased staining of resin composites when used with tea [13].

Resistance to staining is desirable to maintain the esthetic appearance of dental restorations. Rough surfaces may be discolored by adsorption of stains, although there is not always a correlation between surface roughness and staining [14]. Staining susceptibility of resin composite was not related to extrinsic factors such as surface roughness alone, but to intrinsic factors such as monomer and filler composition as well [15]. Surface sealant might provide improved staining resistance than a polished resin composite by reducing the porosity of the surface and by providing a more thoroughly cured surface [16].

Oxygen is known to inhibit polymerization of resins used for restorative dentistry, which forms oxygen inhibition layer at the interface between resin and air. In the Bisphenol-A glycidyl dimethacrylate (Bis-GMA)-dicarbonate/hydroxyetyl methacrylate (HEMA)-carbonate resin, the oxygen inhibition layer was thinner than 1 μm [17]. A sealant/glaze without forming an oxygen-inhibited layer after curing was introduced [18, 19]. In dental field, most studies for the influence of organic substances on the stain accumulation were focused on the methacrylic resins for denture base. However, dimethacrylate-based composite materials are used for the filling of teeth, and the surface properties of resin composite are very diverse by the composition of materials [20]. Although the surface properties of methacrylate and filled dimethacrylate cannot be the same, basic properties would be similar [21]. Though there have been studies on the combined effect of organic substance and chlorhexidine on staining of dental substrates [4, 5, 11, 12], there have been few studies on the effect of surface sealant on the staining susceptibility of resin composites after combined treatment with chlorhexidine and tea solution.

The application of surface sealant to the resin composite restoration was intended to fill in surface defects that persist despite polishing, improve marginal integrity and increase resistance to abrasion [22]. Microleakage was prevented when the margin of resin composite restoration was covered with a surface sealant [23], and surface sealants enhanced marginal sealing [24]. The application of surface sealant to resin composite showed increased wear resistance and marginal integrity [25, 26].

The working hypothesis of the present study was that there were significant differences in the staining of resin composites by the brand of resin composite and by the surface sealant application. The objective of this study was to measure the effect of combined treatment with mucin (a substitute for salivary organic substance), chlorhexidine, and tea solution on the staining of resin composites, and to determine the effect of surface sealant (BisCover, Bisco, USA) application on stain accumulation.

2 Material and methods

Four resin composites were studied (Table 1). The shade of Filtek Supreme (FSP) composite was A2E, and that of Gradia Direct (GRD), Simile (SIM) and Vit-l-escence (VIT) was A2. Resin composite was packed into a polytetrafluoroethylene mold (10 mm in diameter and 2 mm in thickness) on a polyethylene film. After packing the composite, another polyethylene film was pressed on the top of the specimen and loaded with a 5 kg load for 3 min to produce a uniform thickness. Specimen was then light cured for 40 s in 3 overlapping areas with a light-curing unit (The Max, Dentsply/Caulk, Milford, DE). The output of the curing light was verified with a radiometer (SDS/Kerr, Orange, CA). Ten specimens were fabricated for each composite. Specimens were polished with a 600-grit silicon carbide paper on one side. For polishing, the specimen was rubbed against a sheet of wet silicon carbide paper for 50 strokes of 15 cm in length. A surface sealant (BisCover**,** Bisco Inc, Schamburg, IL, Lot number 0300007354) was applied in one group (SS group, 5 specimens) and was not applied in the other group (NO group, 5 specimens, control). The composition of BisCover was as follows: 20–50% ethoxylated bisphenol A diacrylate (Bis-EMA), 20–40% urethane acrylate ester and 20–40% polyethyleneglycol diacrylate based on its material safety data sheet. In SS group, the surface of the specimen was etched with 32% H_3PO_4 solution (UNI-ETCH, Bisco Inc) for 15 s, rinsed with distilled water and dried with air syringe. After then, 1 thin coat of sealant was applied, air thinned to distribute evenly and light-cured with a light-curing unit following the manufacturer's instructions.

Treatment procedures consisted of 4 steps. Immersion solutions are listed in Table 2. Amounts of immersion solutions were 15 ml/specimen. Ionic composition of phosphate buffered saline (PBS) used in this study was as follows; 0.01 M phosphate buffer, 0.0027 M potassium chloride and 0.137 M sodium chloride, pH 7.4 at 25°C. The first step was forming an initial pellicle-like layer with mucin (MCP), which was extracted from porcine stomach (Type II). Specimens were immersed in 37°C MCP for 72 h.

Table 1 Resin composites used in this study

Code	Brand name	Composition	Batch number	Manufacturer
FSP	Filtek supreme	Filler: 59.5 vol.% combination of aggregated zirconia/silica cluster filler with primary particle size of 5–20 nm, and a non-agglomerated 20 nm silica filler Resin: Bis-GMA, Bis-EMA, UDMA, TEGDMA	3AF	3M ESPE, St. Paul, MN, USA
GRD	Gradia direct	Filler: 64–65 vol.% microhybrid filler Resin: urethane dimethacrylate co-monomer matrix	0305132	GC America, Alsip, IL, USA
SIM	Simile	Filler: 68 vol.% nanohybrid filler Resin: polycarbonate/Bis-GMA, Bis-GMA, UDMA, HDDMA	77325	Pentron clinical technologies, Wallingford, CT, USA
VIT	Vit-l-escence	Filler: 58 vol.% microhybrid filler of 0.7 µm Resin: Bis-GMA based	56V4	Ultradent, South Jordan, UT, USA

The second step was chemical alteration of adsorbed mucin with chlorhexidine (CHX) [27]. Specimens were immersed in CHX for 24 h. After the first step and after the second step, specimens were dried for 2 h in an oven at 37°C to make stable coatings. After 2 h drying, surface coating became dried and looked stable.

The third step was the determination of the influence of tea solution (TEA) on staining. Specimens were immersed in TEA for 72 h, and color was measured at 24 h and 72 h after drying for 2 h in an oven at 37°C because deposits could be removed during blot drying.

The fourth step was detaching and dissolving of deposits from the specimen. Specimens were ultrasonically cleaned for 1 h in distilled water, and then immersed in PBS for 72 h. After then color was measured after blot drying.

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, GretagMacBeth Instruments Corp, New Windsor, NY) after blot drying. An ultraviolet component of illumination was included, and the spectral component was excluded (SCE mode) [28]. The aperture size was 3×8 mm. Illuminating and viewing configuration were CIE diffuse/8° geometry. Measurements were repeated three times for each condition of specimen.

Color difference was calculated based on CIELAB as $\Delta E^*_{ab} = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$. A value of ΔE^*_{ab} of 3.3 was considered clinically acceptable in the present study [29]. Changes in value (ΔL^*, lightness), red-green parameter (Δa^*), yellow-blue parameter (Δb^*), chroma (ΔC^*_{ab}) and hue (ΔH^*_{ab}) after immersion in TEA for 72 h were determined as a function of the brand of resin composite and the surface sealant application. ΔL^* was calculated as 'CIE L^* value after immersion in TEA for 72 h—CIE L^* value at baseline', and other differences were calculated with the same method. Chroma change was calculated as $\Delta C^*_{ab} = [(\Delta a^*)^2 + (\Delta b^{*2})]^{1/2}$, and hue change was calculated as $\Delta H^*_{ab} = [(\Delta E^*_{ab})^2 - (\Delta L^*)^2 - (\Delta C^*_{ab})^2]^{1/2}$ [28].

Surface gloss was measured with a surface gloss meter (Novo-Curve Small Area Glossmeter, Rhopoint

Table 2 Immersion solutions

Immersion solution	Concentration	Batch number	Manufacturer
Phosphate buffered saline (PBS)	0.01 M	014K8210	Sigma, St. Louis, MO, USA
Mucin in PBS (MCP)	3 g/l in PBS	113K1012	Sigma, St. Louis, MO, USA
Chlorhexidine digluconate (CHX)	0.2 wt.% in PBS	307–826	STERIS Co., St. Louis, MO, USA
Tea solution (TEA)	10 g/l in distilled water	3K25SB052	Lipton, Englewood Cliffs, NJ, USA

Instrumentation Ltd, East Sussex, UK) for the polished and surface sealant applied specimens to check whether the surface sealant made a uniform coating.

Changes in color and color parameters after immersion in tea solution for 24 h and 72 h, and after cleaning were analyzed by a repeated measures, 2-way analysis of variance (ANOVA) with the independent variables of the brand of resin composite and surface sealant application, and means were compared with Scheffe's multiple comparison test (SPSS 11.0, SPSS Inc, Chicago, IL) at the 0.05 level of significance [30].

3 Results

Color changes (ΔE^*_{ab}) from the baseline after immersion in tea solution for 24 h, 72 h (third step) and ultrasonic cleaning for 1 h and immersion in PBS for 72 h (fourth step; cleaning) are shown in Fig. 1. The range of ΔE^*_{ab} values of four resin composites after immersion in tea solution for 24 h was 9.6–14.9 for NO (no sealant) group, and 9.3–17.0 for SS (surface sealant) group. That after immersion for 72 h was 11.4–21.1 for NO group, and 10.5–19.6 for SS group. That after cleaning was 2.4–10.0 for NO group, and 2.7–8.3 for SS group. Cleaning effect (color changes after cleaning compared to those after immersion in TEA for 72 h) was high. The range of cleaning effect in ΔE^*_{ab} units for NO group was 7.0–9.3, and that for SS group was 7.0–10.4.

Based on repeated measures, 2-way ANOVA, ΔE^*_{ab} value was influenced by the immersion period in tea solution and cleaning procedure (measurements at three points with repeated model), and was influenced by the brand of resin composites ($p < 0.05$), but was not influenced by the surface sealant application ($p = 0.31$).

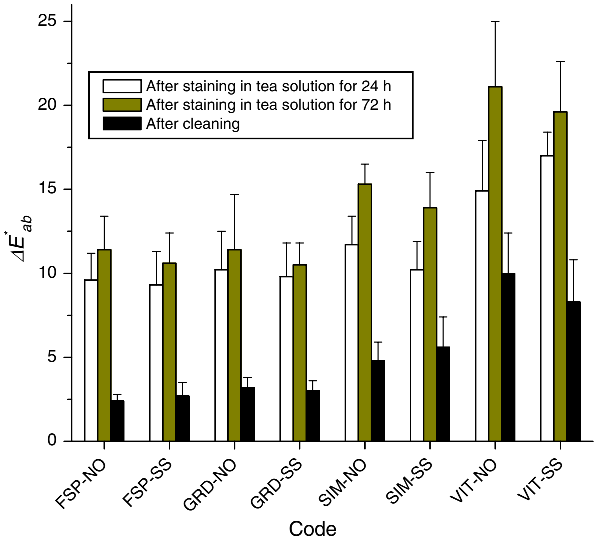

Fig. 1 Color changes after immersion in tea solution and cleaning

In NO group after staining in tea solution for 72 h, VIT showed the highest color change (ΔE^*_{ab}) followed by SIM, GRD and FSP (VIT > SIM > GRD = FSP, $p = 0.05$). In SS group after staining in tea solution for 72 h, the same trend was observed. After cleansing, the same trends by the brand of resin composites were observed regardless of surface sealant application.

Changes in red-green parameter (Δa^*), yellow-blue parameter (Δb^*) and hue (ΔH^*_{ab}) values after immersion in tea solution for 72 h from the baseline are listed in Table 3. Based on repeated measures, 2-way ANOVA, ΔL^* value was influenced by the immersion period in tea solution and cleaning procedure, and was influenced by the brand of resin composites and the surface sealant application ($p < 0.05$). Δa^*, Δb^*, ΔC^* and ΔH^* values were influenced by the immersion period in tea solution and cleaning procedure, and were influenced by the brand of resin composites, but were not influenced by the surface sealant application ($p = 0.75$, 0.18, 0.26 and 0.25, respectively). Comparing the brand of resin composites, VIT showed the highest change in Δa^*, Δb^*, ΔC^* and ΔH^* values, followed by SIM, GRD and FSP ($p = 0.05$, Table 3).

Combined vectorial movement of lightness (CIE L^*) and chroma (C^*_{ab}) after immersion in tea solution for

Table 3 Δa^*, Δb^* and ΔH^*_{ab} values after immersion in tea solution for 72 h from the baseline

Code	TX[a]	Δa^{*b}	Δb^*	ΔH^*_{ab}
FSP (1)[c]	NO	4.0 (1.0)[d]	7.9 (1.6)	0.9 (0.4)
	SS	3.8 (0.7)	8.7 (1.4)	0.8 (0.4)
GRD (2)	NO	3.8 (1.5)	7.7 (2.8)	1.7 (0.6)
	SS	3.9 (0.6)	8.2 (0.9)	1.7 (0.3)
SIM (3)	NO	5.4 (0.5)	10.2 (1.0)	2.2 (0.4)
	SS	5.3 (0.9)	9.3 (1.0)	2.7 (0.3)
VIT (4)	NO	8.3 (1.8)	12.9 (1.4)	5.2 (0.9)
	SS	9.3 (2.6)	11.2 (2.3)	6.0 (1.8)
DG	NO	2 < 4	2.1 < 3 < 4	1 < 2.3 < 4
	SS	1.2.3 < 4	2.1.3 < 4	1 < 3<4

[a] TX = surface treatment, NO means no-treatment after polishing with 600-grit silicon carbide paper, and SS means surface sealant applied

[b] Δa^*, Δb^* and ΔH^*_{ab} mean the changes in CIE a^*, CIE b^*, and hue from the baseline. Δa^* = CIE a^* value after immersion in solution for 48 h–CIE a^* value at the baseline, and so on

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

[d] Standard deviations are in parentheses

72 h from the baseline are shown in Fig. 2. Arrow tails indicate the values at the baseline, and arrow heads indicate the values after staining. After staining, lightness decreased (location of arrow head was moved to lower CIE L^* value compared to that of arrow tail in x-axis) and chroma increased for all composites (location of arrow head was moved to higher chroma value compared to that of arrow tail in y-axis). CIE L^* value at the baseline was 53.4–65.4, which shifted to 48.7–53.0 after staining. C^*_{ab} value at baseline was 7.0–10.2, which shifted to 16.9–26.0 after staining. There was no obvious difference in the changes of lightness and chroma after staining by the surface sealant application. After the application of surface sealant, all the four composites showed small differences in lightness and chroma from those of control group (compare arrow tails of the same composite).

4 Discussion

The working hypothesis of the present study was partially accepted because there was significant difference in the staining by the brand of resin composites ($p < 0.05$), but the effect of surface sealant was insignificant in most cases ($p > 0.05$). In the present study, the changes in color/color parameters after immersion in tea solution and after cleaning were the highest in VIT. These results might be related to the composition of resin matrix. VIT was mainly composed of Bis-GMA resin, but other three resin composites contained urethane-based resin. It has been reported that urethane-modified Bis-GMA based monomer system showed a 10-fold reduction in the release of a Bis-GMA derived product [31], which reflects the surface degradation of resin composites. Therefore, high staining in Bis-GMA based composite might be related with high surface degradation. Filler content of VIT was 58 vol.%, which was lower than those of other resin composites. This might also have influenced high staining. Further studies on these subjects are recommended.

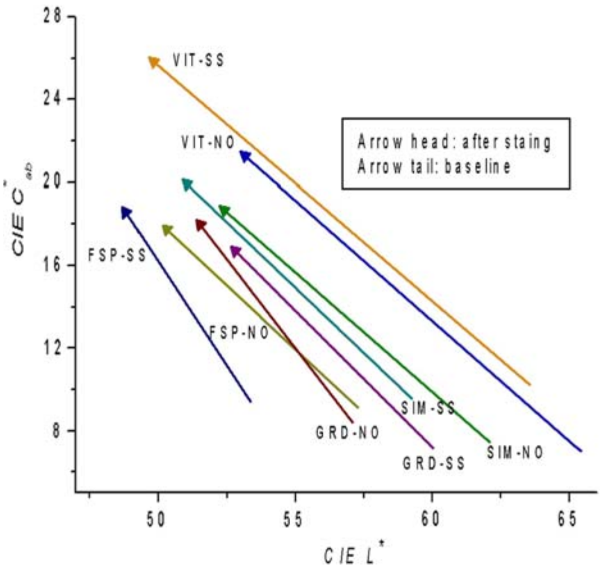

Fig. 2 Vectorial Movement of CIE L^* and C^*_{ab} values after immersion in tea solution for 72 h

The lightness (CIE L^*) and chroma (C^*_{ab}) of NO-group and SS-group showed significant difference (Fig. 2). After surface sealant application, lightness decreased in FSP and chroma increased in SIM and VIT. The changes in lightness and chroma varied by the resin composites. The direction of change seemed to be related to the relative lightness and chroma of resin composites themselves and the surface sealant. For FSP, shade used was an enamel shade that possesses larger translucency, which might also have favored the decrease of the lightness after the application of surface sealant. Therefore general trends could not be concluded.

In the preset study, 600-grit polishing was performed to simulate the rough surface after finishing. If polished with finer particle abrasives, the amount of stain accumulation in the control group might have been changed. However, the results of the present study have clinical implications in the case when roughly finished or worn-off surface was treated or not treated with a surface sealant. Rough surface might increase the bonding performance between surface sealant and resin composite. After the first and the second steps, specimens were dried to provide stable coatings. In vivo condition, immersion and drying cycles in saliva or chlorhexidine are performed with a short time period schedule; however, simulation of these steps could not be performed. Therefore, long time immersion and drying cycles were used in the present study.

In the present study, mucin was used to lay down a pellicle layer. But practically, salivary pellicle contains some mucin but only a relatively small proportion [32]. Additionally, it was different from clinical condition that salivary pellicle was developed for 72 h without being impacted by chromogens or other agents such as dietary acid, all of which would affect the final layer. These differences, compared to clinical condition, should be considered in extrapolating the result of the present study. Additionally instead of mucin in PBS, artificial saliva with organic substances such as proline rich protein may be used [33].

Chlorhexidine was made up in PBS in the present study because PBS was a substitute for saliva to mimic its buffering effect. However, this might have influenced the activity of chlorhexidine by the production of chlorhexidine phosphate.

Color changes of a provisional resin were measured after storage in three staining solutions (black coffee, cranberry juice and red wine) to determine stain resistance after treatment with three surface sealants. ΔE^*_{ab} values were from 1.1 to 6.5 after immersion in staining solutions for 72 h, and varied by the surface sealants applied [16]. In the present study, although not directly comparable solutions were used, color changes by staining in tea solution were higher than those of the previous study probably because mucin was coated and chemical alteration by chlorhexidine was performed on the specimens before the application of staining solution. Therefore, more obvious comparison by the resin composite and surface sealant application was possible. However, color changes after staining were not acceptable regardless of the resin composites ($\Delta E^*_{ab} > 3.3$).

The application of surface sealant to the surface of resin composite resulted in a noticeable improvement in the surface texture based on scanning electron microscopy and the thickness ranged from 0 to 70 µm [22]. The capacity of surface sealant to mask surface defects of resin composite was observed, but it was difficult to obtain a regular surface with liquid resin [22]. This report may support our assumption that surface properties of specimens were mainly influenced by characteristics of resin composites; however, further study is needed for this observation.

Resin composites were shown to be susceptible to staining in body as well as on surface [34]. In the present study, based on the surface gloss measurement, gloss was found to vary by the specimen and the area of a specimen even within the same material. Therefore, mean and standard deviation of the measurements were not presented. In some areas surface sealant made a polished mirror effect [higher gloss in Gloss Unit (GU)], smoother than that produced by polishing as described by Bertrand et al. [22]; however, in other areas, the gloss decreased compared to that after polishing. Therefore, surface sealant should be applied carefully, or further improvement in properties of sealant should be tried.

Surface sealant used in the present study contains 20–50% of Bis-EMA. Bis-EMA was regarded to contribute to higher staining in surface sealant coated resin [16]. However, in the present study, there was no significant increase of staining in SS group. There might be two reasons for this result. One might be the different mechanism of staining of the present study compared to the previous study, and the other might be modified chemical properties of the sealant used in the present study.

The retention of surface sealant on polished composite surfaces was investigated previously. Sections of three differently resin-coated composites were prepared in vitro and examined by scanning electron microscopy. All specimens showed interrupted interfaces (interstices) between glaze and composite [35]. This report might support the higher variations of color changes of the present study (Fig. 1) than other color studies [36].

In the present study, color changes after immersion in tea solution for 24 h or 72 h were not acceptable regardless of the composites and surface sealant application ($\Delta E^*_{ab} = 9.3$–17.0, and 10.5–21.1, respectively). Color changes after cleaning from the baseline were acceptable in FSP and GRD ($\Delta E^*_{ab} = 2.4$–3.2), but were not acceptable in SIM and VIT ($\Delta E^*_{ab} = 4.8$–10) regardless of the surface sealant application (critical $\Delta E^*_{ab} = 3.3$) [29]. Cleaning effect (color change after cleaning compared to that after immersion in TEA for 72 h) was not different by the composite and surface sealant application. After staining, lightness decreased and chroma (C^*_{ab}), CIE a^* and b^* values increased for all the composites, and surface sealant did not influence the changes in these color parameters. Therefore, although the color changes by staining varied by the composites, surface sealant did not influence the changes in color and color parameters by staining.

Within the limitations of the present study, color changes of resin composites were not acceptable after staining ($\Delta E^*_{ab} > 3.3$). There was no significant difference in the changes of color and color parameters by the surface treatment except for a few combinations of color parameters and resin composites.

References

1. R. I. VOGEL, *J. Oral. Med.* **30** (1975) 99
2. A. WATTS and M. ADDY, *Br. Dent. J.* **190** (2001) 309
3. H. NORDBO, H. M. ERIKSEN, G. ROLLA, A. ATTRAMADAL and H. SOLHEIM, *Scand. J. Dent. Res.* **90** (1982) 117
4. H. NORDBO, A. ATTRAMADAL and H. M. ERIKSEN, *J. Prosthet. Dent.* **49** (1983) 126
5. H. PONTEFRACT, M. COURTNEY, S. SMITH, R. G. NEWCOMBE and M. ADDY, *J. Clin. Periodontol.* **31** (2004) 1
6. C. SIPAHI, N. ANIL and E. BAYRAMLI, *J. Dent.* **29** (2001) 197
7. D. C. JAGGER, L. AL-AKHAZAM, A. HARRISON and J. S. REES, *Int. J. Prosthodont.* **15** (2002) 549

8. S. P. HUMPHREY and R. T. WILLIAMSON, *J. Prosthet. Dent.* **85** (2001) 162
9. L. A. TABAK, M. J. LEVINE, I. D. MANDEL and S. A. ELLISON, *J. Oral. Pathol.* **11** (1982) 1
10. S. G. KENG and M. LIM, *Quintessence Int.* **2**7 (1996) 341
11. M. ADDY and S. W. PRAYITNO, *J. Periodontol.* **5**1 (1980) 39
12. A. LEARD and M. ADDY, *J. Clin. Periodontol.* **2**4 (1997) 115
13. Z. A. KHOKHAR, M. E. RAZOOG and P. YAMAN, *Quintessence Int.* **2**2 (1991) 733
14. R. J. SMALES and D. C. GERKE, *Am. J. Dent.* **5** (1992) 208
15. A. F. REIS, M. GIANNINI, J. R. LOVADINO and G. M. AMBROSANO, *Dent. Mater.* **1**9 (2003) 12
16. P. G. DORAY, M. S. ELDIWANY and J. M. POWERS, *J. Esthet. Restor. Dent.* **1**5 (2003) 244
17. W. J. FINGER, K. S. LEE and W. PODSZUN, *Dent. Mater.* **1**2 (1996) 256
18. N. BARGHI and C. ALEXANDER, *Compend. Contin. Educ. Dent.* **2**4(8 Suppl) (2003) 30
19. B. B. SUH, *Compend. Contin. Educ. Dent.* **2**4(8 Suppl) (2003) 27
20. H. LU, L. B. ROEDER and J. M. POWERS, *J. Esthet. Restor. Dent.* **17** (2005) 102
21. A. U. YAP, M. K. MAH, C. P. LYE and P. L. LOH, *Dent. Mater.* **2**0 (2004) 370
22. M. F. BERTRAND, E. LEFORESTIER, M. MULLER, M. L. LUPI-PEGURIER and M. BOLLA *J. Biomed. Mater. Res.* **5**3 (2000) 658
23. K. ITOHM, M. IWAKU, T. FUSAYAMA, *J. Prosthet. Dent.* **4**5 (1981) 606
24. R. P. RAMOS, D. T. CHIMELLO, M. A. CHINELATTI, R. G. DIBB and J. MONDELLI, *Oper. Dent.* **2**5 (2000) 448
25. G. L. DICKINSON, K. F. LEINFELDER, R. B. MAZER and C. M. RUSSEL, *J. Am. Dent. Assoc.* **1**21 (1990) 251
26. K. KAWAI and K. F. LEINFELDER, *Dent. Mater.* **9** (1993) 108
27. H. NORDBO, A. ATTRAMADAL and H. M. ERIKSEN, *Scand. J. Dent. Res.* **9**1 (1983) 182
28. Cie (International Commission On Illumination) In "Colorimetry—Technical Report", Cie Pub. No.15, 2nd edn. (Cie Central Bureau, Vienna, 1994)
29. I. E. RUYTER, K. NILNER and B. MOLLER, *Dent. Mater.* **3** (1987) 246
30. G. R. Norman and D. L. Streiner, In "Biostatistics", (Mosby, St. Louis, 1994)
31. Y. FINER and J. P. SANTERRE, *J. Biomed. Mater. Res. A.* **6**9 (2004) 233
32. J. LI, E. J. HELMERHORST, R. F. TROXLER and F. G. OPPENHEIM, *J. Dent. Res.* **8**3 (2004) 60
33. H. S. KIM and D. D. MILLER, *J. Nutr.* **1**35 (2005) 532
34. G. L. LINGARD, E. H. DAVIES and J. A. VON FRAUNHOFER, *J. Dent.* **6** (1978) 247
35. P. LAMBRECHTS and G. VANHERLE, *J. Oral. Rehabil.* **9** (1982) 107
36. Y. K. LEE, B. S. LIM, S. H. RHEE, H. C. YANG and J. M. POWERS, *J. Biomed. Mater. Res. B.* **7**1 (2004) 16