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Effect of Application Phase of Different Adhesive Systems on Coronal Discoloration Caused by Endoseal MTA: An In-vitro Study

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ABSTRACT

Objectives: This *in-vitro* study evaluated the effect of different application phases of dentin bonding agents (DBA) on preventing crown discoloration caused by a calcium silicate-based root canal sealer.
Materials and Methods: Samples were divided into three main groups: Pre-preparation, Pre-obturation, and Pre-preparation/Pre-obturation, with each group further divided based on adhesive systems. DBA was applied before irrigation in the Pre-preparation, after irrigation in the Pre-obturation, and both before and after irrigation in the Pre-preparation/Pre-obturation groups. After applying the sealer, color measurements were recorded at baseline (T0), 1 month (T1), and 6 months (T2).
Results: Findings showed that application phase, evaluation period and the interaction between these factors significantly affected discoloration. The lowest color change was observed between T1-T0, with significant differences between evaluation periods. The highest color change occurred in the Pre-preparation and control groups at T2-T0 compared to other groups. However, all groups showed color changes above the clinical acceptability (p>1.8) and perceptibility thresholds (p>0.8).
Conclusion: The application phase of DBA and evaluation period significantly influenced crown discoloration, with the greatest changes observed during the pre-preparation phase, emphasizing the critical role of procedural timing in minimizing aesthetic impacts.

Keywords: Adhesive system, Calcium silicate- based sealer, Coronal discoloration, Endoseal MTA

Farklı Adeziv Sistemlerin Uygulanma Aşamasının Endoseal MTA'nın Neden Olduğu Koronal Renk Değişikliğine Etkisi: Bir İn-vitro Çalışma

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ÖZ

Amaç: Bu *in-vitro* çalışmada, kalsiyum silikat esaslı kök kanal patının neden olduğu koronal renk değişikliğini önlemede dentin bonding ajanlarının (DBA) farklı uygulanma aşamalarının etkisi değerlendirilmiştir.
Gereçler ve Yöntemler: Örnekler üç ana gruba ayrıldı: Preparasyon öncesi, Obturasyon öncesi ve Preparasyon öncesi /Obturasyon öncesi. Her grup daha sonra adeziv sistemlere göre subgruplara ayrıldı. DBA, Preparasyon öncesi grubunda irrigasyondan önce; Obturasyon öncesi grubunda irrigasyondan sonra ve Preparasyon öncesi /Obturasyon öncesi gruplarında irrigasyondan önce ve sonra uygulandı. Kanal patı uygulandıktan sonra, renk ölçümleri başlangıçta (T0), 1 ayda (T1) ve 6 ayda (T2) kaydedildi.
Bulgular: Analiz sonuçları, uygulama aşamasının, değerlendirme zamanının ve bu faktörler arasındaki etkileşimin renk değişikliğini önemli ölçüde etkilediğini gösterdi. En düşük renk değişimi T1-T0 arasında gözlendi ve değerlendirme periyodları arasında önemli farklılıklar vardı. T2-T0 değerlendirme periyodunda, en fazla renk değişimi diğer gruplara kıyasla Preparasyon öncesi ve kontrol gruplarında meydana geldi. Ancak tüm gruplar klinik kabul edilebilirlik (p>1.8) ve algılanabilirlik eşiklerinin (p>0.8) üzerinde renk değişimleri gösterdi.
Sonuçlar: DBA uygulanma aşaması ve değerlendirme zamanı krondaki renklenmeyi önemli ölçüde etkiledi, en fazla renk değişimi Preparasyon öncesi grubunda gözlendi ve bu da estetik etkileri en aza indirmede prosedür zamanlamasının kritik rolünü vurguladı.

Anahtar Kelimeler: Adeziv sistem, Endoseal MTA, Kalsiyum silikat bazlı kanal patı, Koronal renk değişikliği.

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Introduction

Discoloration of teeth following endodontic procedures presents a frequent aesthetic concern, especially in anterior teeth and can lead to an aesthetic problem that can negatively affect the patient's quality of life.[1] The primary reasons for tooth discoloration associated with endodontic treatment from residual necrotic pulp tissue, intra-canal medicaments, irrigant solutions (such as the interaction between sodium hypochlorite and chlorhexidine), and filling materials.[2] Specific components within these materials, such as eugenol, bismuth oxide, or silver, along with chemical reactions triggered by contact with irrigants, dentin collagen, or blood, can contribute to the discoloration.[3,4] This darkened pigments can permeate through the hard tissue. Additionally, particles of these materials can infiltrate the dentinal tubules, leading to long-term discoloration.[5]

Bismuth oxide, utilized as a radiopacifier in certain dental cements or sealers[6], triggers a grayish discoloration when it interacts with dentin collagen.[7] Additionally, when exposed to substances such as sodium hypochlorite (NaOCl), chlorhexidine, blood, or glutaraldehyde, it forms dark compounds.[8] These compounds can permeate through dentinal tubules, potentially reaching the dental crown and resulting in a visible alteration in color. Understanding these chemical reactions is crucial for dental professionals in selecting and using materials effectively, ensuring optimal outcomes for patients.

In scientific literature, dental discoloration has been extensively documented, particularly with certain types of dental cements. Resin-based cements, notably AH26, as well as eugenol-based cements, and calcium-silicate based sealers containing bismuth oxide as a radiopacifier, have been associated with heightened incidences of discoloration.[9-11] Endoseal MTA is a pre-mixed form of root canal sealer based on pozzolan cement in a syringe. It has superior physical and biological characteristics of MTA. Endoseal MTA contains bismuth oxide as a radiopacifier could be resulting in crown discoloration.[12]

The effectiveness of a dentin bonding agent (DBA) in sealing the dentinal tubules within the pulp chamber can play a significant role in reducing tooth discoloration caused by various factors.[13,14] By properly sealing these tubules, the penetration of substances that cause discoloration, such as those mentioned earlier like blood, NaOCl, or chlorhexidine, can be minimized.[13,15] This preventive measure helps maintain the natural color of the tooth and preserves its aesthetic appearance.

Different methods such as Erbium-doped yttrium aluminum garnet (Er:YAG) laser, Neodymium-doped yttrium aluminum garnet (Nd:YAG) laser, desensitizers, and DBA are utilized to achieve dentinal tubule occlusion and prevent MTA-induced discoloration.[16] However, considering its ease of access and cost-effectiveness, the use of an adhesive is recommended for dentin tubule occlusion.[17] In literature, although there are studies in the literature indicating that dentin bonding agents reduce discoloration when using MTA-based root sealers or cements.[14,18,19] To the best of our knowledge, no study has investigated different application phases and adhesive application strategies. In the present *in-vitro* study, the aim is to analyze the effect of the application phase of different adhesive systems on preventing coronal discoloration (ΔE_{00}) caused by calcium silicate-based canal sealer (Endoseal MTA), evaluated by spectrophotometry, over a period of 6 months. The null hypothesis is that all groups will have ΔE_{00} values below the level of acceptability.

Material and Method

The sample size for the study was determined using G*Power software (Version 3.1.9.4, Heinrich Heine University, Düsseldorf, Germany). A significance level (α) of 0.05 and a statistical power of 90% were used as the parameters for the calculation, resulting in a minimum required sample size of 5 participants per group. To avoid a potential 20% dropout rate, the sample size was increased to 10 subjects per group, resulting in a total of 96 subjects for the study.

The samples were obtained from animals killed for commercial reasons. The study adhered to the principles outlined in the Guide for the Care and Use of Laboratory Animals (www.nap.edu/catalog/5140.html), ensuring the protection of animal rights. Ethical approval was obtained from the institutional ethics committee (Decision no: 2024/39). Ninety-six bovine incisor teeth with similar crown and root dimensions, free from white spots, caries, cracks, or other defects, were selected and disinfected by immersion in a 0.01% thymol solution. Before use, any residual soft tissues and periodontal fibers on the root surfaces were carefully removed using an ultrasonic device and polished with a pumice slurry and brush. The prepared teeth were then stored in a sterile saline solution at room temperature until procedure started.

Experimental Setup

The apical portion of each specimen was resected 3 mm below the labial cementoenamel junction to standardize crown length, using high-speed diamond discs under water cooling. The pulp was removed using a spoon excavator and the pulp chambers were chemo-mechanically debrided using 6# Gates Glidden drills and 2.5% NaOCl through the apical access.[10] Subsequently, the crown pulp chambers were irrigated with 20 mL of 5.25% NaOCl for 5 minutes and dried with paper points.

The specimens were randomly assigned to three main groups regarding by application phase of dentin bonding agents (DBAs): Pre- preparation; Pre- obturation; and Pre-preparation/Pre- obturation (n=30). Each group was then randomly divided into three experimental groups (n=10) according to the adhesive systems (One-step Self etch (all in one); Two-step self-etch, and Two-step total etch). The adhesive systems manufacturer's information, composition, and application procedures are presented in Table 1.

Table 1. Manufacturer and composition of root canal sealer and adhesive materials used in the study.

Material	Manufacturer	Composition
Endoseal MTA	Maruchi, Wonju, Korea	Calcium silicates, Calcium aluminates, Calcium aluminoferrite,Calcium sulfates, Radiopacifier, Thickening agents
Clearfill SE Bond	Kuraray, Okayama, Japan	Primer: MDP, HEMA, Hydrophilic dimethacrylate, water,Adhesive: MDP, bis-GMA, HEMA, dimetachrylate, silanated colloidal silica
Primer&Bond® NT	Dentsply Sirona Konstanz, Germany	Di- und tri-methacrylates, PENTA, nanofillers, photoinitiators, stabilizers, acetylamine-hydrofluoride, aceton
Tokuyama Bond Force II	Tokuyama Dental, Tokyo, Japan	Self-reinforcing 10-MDP (10-methacryloyloxydecyl dihydrogen phosphate), Bis-GMA, TEGDMA, HEMA, alcohol, water

MDP: methacryloyloxydecyl dihydrogen phosphate; HEMA: 2-hydroxyethyl methacrylate; Bis-GMA: bisphenol-adiglycidyl methacrylate; PENTA: dipentaerythritol penta-acrylate monophosphate TEGDMA: triethyleneglycol dimethacrylate.

In the pre-preparation group, irrigation protocol was applied after DBA application; in the pre-obturation group, DBA application was performed after irrigation protocol. In the Pre-preparation/Pre-obturation group, DBA was applied before and after irrigation. Endoseal MTA (Maruchi, Wonju, Korea) was then injected into the pulp chambers via cervical access. For standardization, the same operator (M.B.) performed all application procedure. The all specimens stored at 37ºC and 95% humidity until their respective evaluation times were reached.

Color Measurement

The color measurement of specimens was performed by using digital spectrophotometry (Vita Easyshade V, Vita Zahnfabrik, Bad Säckingen, Germany) setting to "tooth mode" at different intervals (T0=Baseline; T1=1-Month; T2=6-Months). For standardization, the same operator (A. T. E. A.) performed all color measurements. Color measurements were obtained from the buccal surfaces of crowns. The "L*, C*, H*, a*, and b*" values were measured separately on a black background under constant laboratory illumination. These values were measured thrice and the mean values were recorded. The spectrophotometer was recalibrated according to the manufacturer's instructions after nine measurements. Color changes (ΔE_{00}) were calculated using the CIEDE2000 formula as follows:

$$\Delta E_{00} = [(\frac{\Delta L}{k_L S_L})^2 + (\frac{\Delta C}{k_C S_C})^2 + (\frac{\Delta H}{k_H S_H})^2 + R_T(\frac{\Delta C}{k_C S_C})(\frac{\Delta H}{k_H S_H})\ ]^{1/2}$$

Tolerance encompasses two components: firstly, 50:50 perceptibility, which denotes the color difference detectable by 50% of individuals; and secondly, 50:50 acceptability, which indicates the color difference that 50% of individuals find tolerable. For ΔE_{00}, the acceptability threshold is defined as a value below 1.8 units, whereas the perceptibility threshold is indicated at a value below 0.8 units.[20]

Statistical Analysis

All statistical analyses were performed using the Statistical Package for the Social Sciences software (SPSS Inc., Chicago, IL, USA). The Shapiro-Wilk test was used to analyze the normality of the data. The data were analyzed using three-way ANOVA and Tukey post hoc tests and Bonferroni correction. 'p' value of .05 was considered significant.

Results

The application phase, adhesive systems, ΔE_{00} evaluation periods, and their interactions were analyzed regarding color changes. It was found that the application phase, ΔE_{00} evaluation period, and the interaction between these two factors significantly affected color change ($p<0.001$, $p<0.001$, and $p=0.011$, respectively) (Table 2). The lowest color change was observed during $\Delta E_{00}1$ (T1-T0), with significant differences noted across the other ΔE_{00} evaluation periods ($p<0.05$) (Table 3). In the $\Delta E_{00}3$ (T2-T0) evaluation period, the greatest color change occurred in the pre-preparation phase and the positive control group, showing a more notable difference compared to the pre-obturation and pre-preparation/pre-obturation phases ($p<0.05$) (Figure 1). No significant differences were observed between the adhesive systems and the control group ($p = 0.678$). Nonetheless, the color change of all adhesive systems exceeded the AT threshold (>1.8) and the PT threshold (>0.8).

Table 2: Linear model analysis of application phase, adhesive system and ΔE_{00} evaluation period factors.

	Wald Chi-Square	df	Sig.
Application phase	78.508	2	**< 0.001**
Adhesive system	1.584	2	0.908
ΔE_{00} evaluation period	197.294	2	**< 0.001**
Application phase*Adhesive system	9.528	4	0.678
Application phase*ΔE_{00} evaluation period	32.042	4	**0.011**
Adhesive system*ΔE_{00} evaluation period	4.366	4	0.900
Application phase*Adhesive system*ΔE_{00} evaluation period	6.646	8	0.917

Bold characters indicate significantly effective factors on color change values.

Table 3: Three-way Anova comparisons of discoloration values of the application phase, adhesive system and the ΔE_{00} evaluation period ($\Delta E_{00}1$, $\Delta E_{00}2$, and $\Delta E_{00}3$).

		Adhesive Systems			
	Application Phase	**All in One**	**Self etch**	**Total etch**	**Total**
$\Delta E_{00}1$(T1-T0)	Pre- preparation	4.11 ± 3.29	3.48 ± 1.24	1.90 ± 1.08	3.16 ± 2.19
	Pre- obturation	2.23 ± 1.01	2.63 ± 0.93	2.21 ± 0.87	2.36 ± 0.89
	Pre- preparation/Pre- obturation	3.82 ± 2.68	1.02 ± 0.50	3.25 ± 2.15	2.70 ± 2.24
	Total	3.39 ± 2.49	2.38 ± 1.37	2.45 ± 1.49	3.86 ± 4.12^{A}
$\Delta E_{00}2$ (T2-T1)	Pre- preparation	5.40 ± 4.01	8.89 ± 8.25	6.96 ± 5.5	7.08 ± 5.91
	Pre- obturation	7.02 ± 1.87	5.98 ± 2.77	7.76 ± 1.39	6.92 ± 2.08
	Pre- preparation/Pre- obturation	5.14 ± 3.24	3.80 ± 1.62	5.06 ± 3.16	4.67 ± 2.65
	Total	5.85 ± 3.06	6.22 ± 5.20	6.59 ± 3.66	6.06 ± 3.48^{B}
$\Delta E_{00}3$(T2-T0)	Pre- preparation	9.49 ± 5.76	10.49 ± 11.0	9.48 ± 6.57	9.82 ± 7.52^{a}
	Pre- obturation	6.82 ± 1.98	4.78 ± 1.34	3.53 ± 1.99	5.05 ± 2.18^{b}
	Pre- preparation/Pre- obturation	4.69 ± 1.13	5.30 ± 2.62	5.30 ± 2.74	5.10 ± 2.14^{b}
	Total	7.00 ± 3.88	6.86 ± 6.64	6.10 ± 4.72	7.36± 4.99^{B}
Total	Pre- preparation	6.33 ± 4.77	7.62 ± 8.01	6.11 ± 5.65	6.69 ± 6.19^{C}
	Pre- obturation	5.36 ± 2.77	4.47 ± 2.24	4.50 ± 2.81	4.77 ± 2.59^{C,D}
	Pre- preparation/Pre- obturation	4.55 ± 2.39	3.37 ± 2.48	4.54 ± 2.69	4.15 ± 2.53^{D}
	Total	5.41 ± 3.47^{A}	5.15 ± 5.22^{A}	5.05 ± 3.94^{A}	5.76 ± 4.46

$\Delta E_{00}1$: Discoloration between baseline and at 1-month. $\Delta E_{00}2$: Discoloration between at 1 month and at 6 months. $\Delta E_{00}3$: Discoloration between baseline and at 6- months. ^{A,B}Different uppercase letters represents the differences of color change between time interval. ^{C,D}Different uppercase letters represent the differences of color change between application phase groups in the same column. ^{a,b}Values in the same columns with the different lowercase letters represent significantly different between application phase in $\Delta E_{00}3$ evaluation period. Uppercase letters in the same rows represent the differences of color change between adhesive systems.

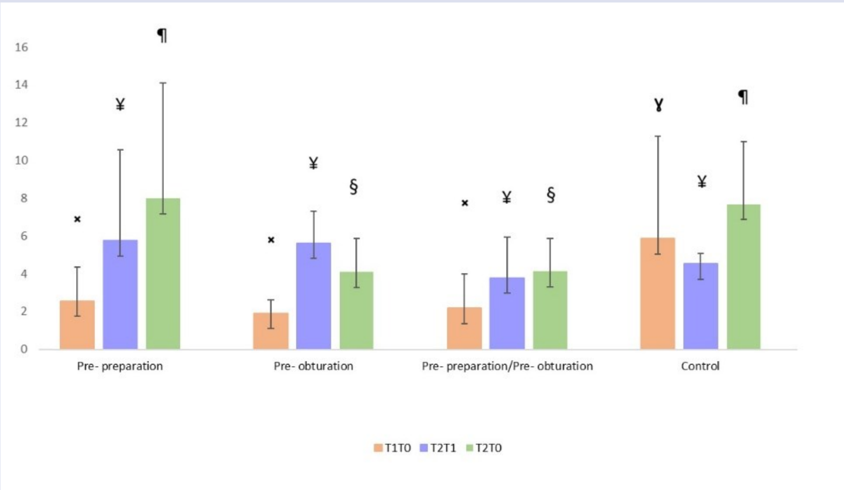

Figure 1. Figure 1: ΔE_{00} values (mean and standard deviation) of the experimental groups at the different interval. T1-T0: Discoloration between baseline and at 1-month. T2-T1: Discoloration between at 1 month and at 6 months. T2-T0: Discoloration between baseline and at 6- months. Values in the same color bar chart with the different sign are significantly different (P < 0.05).

Discussion

Bismuth oxide, the radiopacifier included in the composition of MTA, has been identified as the chemical compound responsible for the discoloration observed with this material.[21] A study demonstrated significant color changes in bismuth oxide when exposed to sodium hypochlorite. [22] To prevent and manage discoloration, prior research has recommended applying a double layer of DBA over the dentin in an access cavity before using MTA.[23] Endoseal MTA contains 47% of a mixture of ZrO_2 and Bi_2O_3 as a radiopacifier.[24] To our knowledge, there was no *in-vitro* assay investigating the effect of different adhesive systems at different application phases —pre-preparation, pre-obturation, and pre-preparation/pre-obturation— on preventing coronal discoloration caused by calcium silicate-based canal sealer (Endoseal MTA) was evaluated.

Discoloration can begin at different times depending on the study and materials used, with some MTA-based products which contains bismuth oxide showing discoloration within one day and others within a range of up to six months.[25] Therefore, in the present study, color was assessed one month after placement, then at six months. The null hypothesis was rejected, since the minimal color change was detected in ΔE_{00}1(T1-T0), with significant differences noted between this period and the other ΔE_{00} evaluation periods (T2-T0; T2-T1). On the other hand, the ΔE_{00} values at the all evaluation periods exceeded both the AT (>1.8) and the PT (>0.8) threshold levels.

In the ΔE_{00}3 (T2-T0) evaluation period, the most significant color change was observed in both the pre-preparation phase and the positive control group. This change was more significant compared to the pre-obturation and pre-preparation/pre-obturation phases. It was well-known that NaOCl adversely effects the bond strength due to its degradation potentional by removing collagen fibrils.[26] In the pre-preparation group, dentinal tubules of pulp chamber was firstly sealed by adhesive systems, then irrigation with 5.25% NaOCl was performed. This may have compromised the bonding agent's ability to prevent discoloration by reducing the durability of the adhesive interface, particularly due to NaOCl's ability to dissolve organic compounds.

Concerning the use of irrigants, the impact of NaOCl on tooth color has been observed in relation to calcium silicate-based cements.[8] This effect is attributed to NaOCl's ability to penetrate between 77 and 300 µm into the dentinal tubules, making it challenging to completely remove.[27] Therefore, in pre-preparation/pre-obturation phase group, adhesive systems were applicated by manual brush before irrigation procedure, pulp chamber was flushed with 5.25% NaOCl in the second step and application of dentin bonding agent was repeated in last step. Calcium silicate-based sealer was then injected into the pulp chamber to ensure it covered all axial walls. The color change observed in this group was statistically less compared to the pre-preparation group. Applying bonding agents twice may help reduce discoloration. However, the color change observed in our study exceeded the clinical threshold. This issue could be related to the gray color of Endoseal MTA and its completely placement in the pulp chamber. Considering that the color change is associated with the properties of these materials and their application techniques, it may be beneficial to explore alternative materials or methods to improve aesthetic outcomes.

Paralel to the results, tooth discoloration in the pre-obturation phase group was significantly less compared to pre-preparation group. Using bonding agents to seal the pulp chamber walls has been recommended. Since the dentinal tubules are obliterated, the bismut oxide cannot penetrate the dentin.[28] The discoloration could be minimized by preventing interaction with NaOCl and bismut oxide.

The obtained results indicate that the adhesive systems used did not have a significant effect on color change. This finding can be considered an important contribution to understanding the impact of adhesives strategies on aesthetic outcomes. In the study, irrigation was performed using 5.25% concentration of NaOCl. It was documented that NaOCl caused destruction to the intratubular surface near the root canal.[29] The use of high concentrations of NaOCl may have increased the severity of discoloration by reducing the bond strength of the adhesives due to changes in the microstructural parameters. Similar to the results of our study, Uslu *et al.*[19] investigated various applications aimed at preventing crown discoloration and indicated that there was no significant difference between the control group and the Clearfil SE Bond group.

Clinically, regardless of the DBA type, less coronal discoloration would result from cases which MTA based cements or intracanal medication used, if DBA were applied to the dentinal walls of the pulp chamber.[30] If DBA is not applied, the discoloration is severe, but when DBA is applied, the crown coloration decreases. However, DBA application may not reduce the color change below clinically acceptable levels.[28]

MTA-based sealers such as EndoSeal MTA widely used in endodontics, has excellent sealing properties and biocompatibility, but it can be associated with discoloration of the dentin over time. The effect of pre-applying a DBA to the dentin walls of the pulp chamber to prevent color changes caused by MTA has been evaluated in several studies. Consistent with the findings of our study, Choi *et al.*[28] used DBA to prevent tooth discoloration caused by MTA. Their study demonstrated that after 12 weeks, there was a significant less discoloration compared to the baseline. Khim *et al.*[14] investigated the effectiveness of DBA application in preventing coronal discoloration caused by various sealers. They concluded that the application of DBA led to lower mean Delta E values. Likewise, the present study demonstrated that the pre-preparation/pre-obturation phase group exhibited less discoloration compared to the positive control group. Therefore, the use of multiple layers and the application of DBA at different phases are recommended for better coverage.

Our study has certain limitations. It is important to consider these limitations when making evaluations. This *in-vitro* study assesses discoloration potential under a "worst-case scenario" by leaving a substantial amount of sealer in direct contact with the axial dentinal walls of the pulp chamber, it should be considered as one of the limitations. Therefore, the findings of this study do not directly reflect the potential for tooth discoloration in real clinical settings. Exposure of a significant amount of sealer to the pulp chamber walls could result in severe discoloration. Furthermore, the impossibility of achieving a completely uniform pulp chamber of bovine teeth is an another of the limitations of the present study.

Conclusions

The pre-obturation and pre-preparation/pre-obturation groups resulted in statistically less color change. However, the color changes observed in all groups were above clinically acceptable level. Considering the limitations of this study, the application phase of the dentin bonding agent may be significant, regardless of the adhesive system used. Applying the bonding agent to the pulp chamber before root canal obturation may help reduce color change. Future research should examine the effects of different adhesive systems and different concentration of NaOCl on long-term color change.

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Conflict of Interest

The authors deny any conflicts of interest related to this study.

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