Effect of irrigation times of sodium ascorbate on bond strengths to bleached enamel using two adhesive systems

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Abstract

Objectives: To evaluate the effect of different irrigation times of 10% sodium ascorbate on microshear bond strengths of two adhesives: Adper™ Single Bond 2 and Clearfil SE Bond, to enamel bleached with 10% carbamide peroxide.

Materials and methods: Sixty sound human premolars were divided into 5 groups regarding to treatment of facial enamel surfaces: 1 no treatment (unbleached enamel), 2 bleached with 10% carbamide peroxide (VivaStyle 10%), the other three groups were bleached and followed by irrigation with 10% sodium ascorbate for 30 sec, 1 min and 10 min respectively. Enamel surface of all specimens were bonded with one of the two adhesives and then restored with a resin composite. The microshear bond strength testing was performed using a universal testing machine at a crosshead speed of 1 mm/min after storage in distilled water at 37°C for 24 hours. The data were analyzed using two-way ANOVA and post-hoc multiple range test ($p<0.05$).

Results: Irrigation with sodium ascorbate for 10 min could reverse the bond strength of bleached enamel in group using Adper™ Single Bond 2 to the same level as that of the control group ($p<0.05$). However, irrigation with sodium ascorbate had no effect on the bond strength of bleached enamel bonded with Clearfil SE Bond.

Conclusion: Pretreatment with sodium ascorbate for 10 min before composite restoration improved the bond strengths of bleached enamel when Adper™ Single Bond 2 was used.

Key words: adhesive, antioxidant, bleaching, bond strength, enamel, irrigation time

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Introduction

At-home tooth bleaching becomes a popular and effective method to remove intrinsic stains from teeth.[1] Bleaching with 10% carbamide peroxide demonstrated no harmful effect to enamel structure.[2,3] Turkun and coworkers[4] revealed slightly increasing of enamel porosity that was reversed within 3 months after treatment. However, the bleaching decreased the immediate bond strength of resin composite to bleached enamel when compared with the unbleached enamel.[5-8] The reduction of bond strength might be due to the presence of residual oxygen, which penetrated into enamel during the bleaching process and accumulated within the enamel structure, inhibited resin polymerization.[9,10] General approach for optimal bond strength is to postpone any bonding procedure for 1-3 weeks after bleaching since the reduction of bond strength has shown to be temporary.[5-7] There were many attempts to improve the reduced bond strengths by applying some antioxidants before restoring the teeth. Some studies showed that the compromised bond strengths were effectively reversed by treating with 10% sodium ascorbate solution before acid etching and adhesive application.[8,11,12] Sodium ascorbate (SA), a sodium salt of ascorbic acid (vitamin C), is relatively safe and widely used as an antioxidant.

Lai et al[8] demonstrated that the reduced bond strength of bleached enamel could be improved by immersed the bleached teeth in 10% sodium ascorbate for one-third of bleaching time to neutralize the effect of bleaching agent prior to acid etching and adhesive application. Some study indicated that 2-hour application time of 10% SA solution could gain the compromised bond strength of bleached enamel.[11] SA has also been used to apply on the NaOCl-treated dentine before the adhesive procedure to reverse the compromised bond strength.[12-14] Morever, the bond strengths of NaOCl-treated dentine can be improved by the short application time of SA. Vongphan and coworkers showed that 10-min[13] or at least 30-sec[14] application time of 10% SA can improve the compromised bond strength of composite restoration to NaOCl-treated dentine when using etch-and-rinse adhesive (Adper™ Single Bond 2). Enamel and dentine are different in structural, chemical compositions and the bonding mechanism. Muraguchi and coworkers[15] revealed that 1 min application of 10% ascorbic acid to bleached bovine enamel could remarkably improve the bond strengths of bleached enamel to the same level of those of non-bleached surfaces. Nevertheless, the bovine teeth have some characteristics that differ from human teeth. The bovine enamel is more porous than human enamel. Moreover, it has thinner crystallites and different prism structure. The bovine enamel has more carbonate concentration which has been reported to be more susceptible to acid attack.[16] So far, there is no study regarding the effect of short application time of SA on reversal of compromised bond strength in bleached human enamel.

Currently, there are two types of adhesive systems that are commonly used in composite restoration; total-etching (etch-and-rinse) and self-etching adhesive system.[17] Cavalli and coworker[6] studied the bond strengths of two adhesive systems; Single Bond; SB (etch-and-rinse adhesive system) and Clearfil SE Bond; SE (self-etching adhesive system) on unbleached and bleached enamel. The 2-step, acid-etch system revealed higher bond strengths in both unbleached and bleached enamel than 2-step self-etching adhesive. The objective of this study was to evaluate the effect of irrigation times of 10% SA solution on the microshear bond strengths of two different adhesive systems on bleached enamel. The null hypothesis is that there were no significant differences among the effect of irrigation times of 10% SA solution on the microshear bond strengths of two adhesive systems to bleached enamel.

Materials and methods

Sixty sound extracted human premolars were used in this study. The methods were performed under the ethical approval by MU-DT/PY-IRB. No. 2010/006.2601. Gross debris was removed and the teeth were kept in 0.1% thymol solution at 4°C for no longer than 1 month after extraction. The teeth were rinsed and placed in distilled water for 24 h before use. All teeth were randomly assigned to five groups of twelve teeth each with Group 1 assigned as the control group (unbleached), the teeth in this group were stored in distilled water 37° C for 1 week with daily change of water and kept in distilled water another 24 h. The teeth in other four groups were bleached with 10% carbide peroxide (10% VivaStyle, Ivoclar Vivadent, Schaan, Liechtenstein) 8 h per day for 1 wk. After daily bleaching process, the teeth were thoroughly rinsed with distilled water for 10 s to remove bleaching agent and stored in distilled water at 37°C. At the end of bleaching regimen (7 days), teeth were rinsed and placed in distilled water at 37°C for 24 h.

Facial enamel surfaces of each tooth were ground with a 600-grit silicon carbide paper (Struers, Ballerup, Denmark) under running water to create a flat enamel surface of 3 x 5 mm. The teeth were then sectioned longitudinally parallel to the flat enamel surface to make a 2 mm thick slab using a low speed diamond saw (Isomet™, Buehler, Lake Bluff, IL, USA) under running water.

*In Group 2, the teeth were only bleached without any application of SA (BL).*The facial ground surface of the enamel slabs in Group 3-5 (BLSA1, BLSA2, BLSA3), were then treated with freshly prepared of 10% SA solution for 30 s, 1 min and 10 min respectively. The rate of irrigation was 0.5 ml/30 s.

For antioxidant solution preparation: 10% SA was freshly prepared by dissolving ascorbic acid sodium salt powder (Sigma-Aldrich, St.Louis, MO, USA.) in distilled water (1:9 ratio by weight) immediately before use.

All enamel slabs in each group were randomly divided into two subgroups of six each for bonding to one of two adhesive systems: two-step etch-and-rinse adhesive (Adper™ Single Bond 2: SB, 3M ESPE, St Paul, MN, USA) or two-step self-etching adhesive (Clearfil SE Bond: SE, Kuraray Med. Inc. Osaka, Japan) (Table 1). The bonding procedures were performed according to the manufacturers' instructions (Table 2). Prior to light activation, two Tygon® Tubes (Saint-Gobain Performance Plastics, Cambridge, UK) with internal diameter 0.8 mm and 1 mm height were placed onto the bonded surface, then light-cured for 10 s with a Heliolux DLX (Vivadent, Schaan, Liechenstein) light-curing unit with a power intensity of 550 mW/cm^2. The light intensity was checked before using. A resin composite; shade A2 Filtek Z250 (3M ESPE, St Paul, MN, USA) was filled into the tube and light cured for 40 s. Two bonded areas were conducted on each specimen. The tubes on the specimen were removed after light curing. The specimens were then placed in distilled water at 37°C for 24 h prior to microshear bond test. Microshear testing was conducted on the universal testing machine (Lloyd Instruments Ltd., Fareham Hanth, UK) with a crosshead speed of 1 mm/min. The bond strength values were calculated and expressed in MPa.

The failure modes were analyzed using a scanning electron microscope (SEM) (JSM 5410LV, JEOL, Tokyo, Japan) at ×100 magnification and classified as adhesive (>75% of the failure occurred between resin and

Table 1 Composition of the materials used in the study

Material	Manufacturer	Composition	Lot No.
Adper™ Single Bond 2	3M ESPE, St. Paul, MN, USA	Etchant: 35% H3PO4 BisGMA, HEMA, dimethacrylates, ethanol, water, nanofiller, a novel photoinitiator system and a methacrylate functional copolymer of polyacrylic and polyitaconic acids	N101454
Clearfil SE Bond	Kuraray Med. Inc., Osaka, Japan.	Primer: MDP, HEMA, hydrophilic dimethacrylate, photo-initiator, water Adhesive: MDP, HEMA, Bis-GMA, hydrophobic dimethacrylate, photinitiators, silanated colloidal silica	81158
VivaStyle 10%	Ivoclar Vivadent AG, Schaan, Principality of Liechtenstein	10% carbamide peroxide, caborpol, glycerine, 0.11% potassium nitrate	065122
Ascorbic acid sodium salt	Sigma-Aldrich, St. Louis, MO, USA	(+)-Sodium L-Ascorbate BioXtra, ≥99.0% NT	11140
Filtek Z250; shade A2	3M ESPE, St Paul, MN, USA	Zirconia silica filler, Triethylene glycol dimethacrylate (TEGDMA), Bis-GMA (bisphenol A diglycidyl ether dimethacrylate) UDMA (Urethane dimethacrylate) Bis-EMA (Bisphenol A polyethylene glycol diether dimethacrylate)	N098240

Table 2 Application procedures of the adhesive systems used in this study

Adhesive resin	Procedure
Adper™ Single Bond 2	Apply etchant 15s to tooth surface. Rinse thoroughly for 10s and blot excess water using a cotton pellet. Apply 2 consecutive coats of adhesive for 15s with gentle agitation. Gently air-thin for 5s. Light cure for 10s.
Clearfil SE Bond	Apply primer with rubbing to the tooth surface and leave in place for 20s. Dry with a mild air stream for 5s. Apply adhesive to the tooth surface and gently air blow. Light cure for 10s

enamel interface), cohesive in enamel (>75% of failure was within the enamel), cohesive failure in resin (>75% of the failure was within the adhesive resin and/or composite), or mixed failure. The failure mode were given as percentage and analyzed using Kruskal-Wallis and chi-square test at a significance level of p< 0.05.

The microshear bond strength data were analyzed for normal distribution and equality of variance using the Kolmogorov-Smirnov's test and Levene's test.The mean bond strengths were statistically analyzed using two-way analysis of variance (ANOVA) to determine the effect of antioxidant applications on bleached enamel. Differences between the groups were further analyzed using post-hoc Dunnett T's multiple range test at p-value=0.05.

Additional twenty premolars were randomly assigned to five groups of four teeth. The teeth in each group were treated before adhesive application as described above. After grinding and treat the facial enamel surfaces, two teeth of each group were applied with one of the two adhesives, then light-cured for 10 s. A two-mm-height composite build-up was performed to the flat enamel bonding area with resin composite; Filtek Z 250 shade A2. The specimens were stored in distilled water for 24 h. After storage time, the root of each tooth was cut off, then the crown was longitudinally sectioned in bucco-lingual direction perpendicular to the bonded interface. Specimens of each group were then embedded in epoxy resin. After 24 h, the embedded specimens were metallurgical polished with silicon carbide paper and diamond paste (DP-Paste, Struers, Ballerup, Denmark) 6 micron, 3 micron down to 1 micron. The specimens were cleaned with distilled water in the ultrasonic machine for 10 min. All polished specimens were subjected for acid etching with 10% phosphoric acid for 5 s, rinsing with distilled water for 30 s and placing in 5.25% sodium hypochorite for 5 min. The specimens were then rinsed with distilled water for 30 s and dried in desiccator for 24 h. The dried specimens were gold-sputter-coated, the resin-enamel interfaces were observed with the scanning electron microscope (JSM 6610LV, JEOL, Tokyo, Japan) at magnification x2,000 and x5,000.

Results

The means and standard deviations of microshear bond strengths are shown in Table 3. The Levene's test for homogeneity of variance demonstrated *p*-value ≥ 0.05. The two-way ANOVA indicated that there were interactions between adhesive systems and irrigation time periods (*p*=0.022). The significant effect to the bond strength was observed only for irrigation time (*p*<0.01). The differences among groups were further analysed using Dunnett T's multiple comparisons.

For SB groups, the bleached group (BL)

Table 3 Means and standard deviations of microshear bond strength in MPa.

μSBS mean±SD (MPa)	SB	SE
Control	24.47 ± 5.28^{a}	26.96 ± 6.86^{a}
Bl	9.35 ± 3.43^{c}	16.99 ± 5.54^{b}
BlSA 1	14.31 ± 7.42^{bc}	15.81 ± 3.91^{b}
BlSA 2	14.13 ± 6.56^{bc}	15.04 ± 6.44^{b}
BlSA 3	24.22 ± 4.99^{a}	18.08 ± 6.67^{b}

Groups identified with the same superscript letter are not significantly different (p>0.05).

demonstrated significant lower bond strength than the unbleached (CONTROL) group (p<0.05). In the antioxidant-treated groups (SA), irrigation with SA 10 min (BLSA3) was significantly effective in reverse the bond strength of bleached enamel to normal (unbleached). There were no significant differences in microshear bond strength among groups of BL, BLSA1 and BLSA2 (p>0.05). The bond strength of BLSA3 group revealed higher than that of BL, BLSA1 and BLSA2 group (p<0.05), but not statistically significant difference from the CONTROL (p>0.05).

For SE groups, the microshear bond strength of resin composite to bleached enamel BL/SE was significant lower than that of unbleached enamel(CONTROL) (p<0.05). For the bleaching groups, sodium ascorbate had no effect on the bond strength of bleached enamel. There were no significant differences of the microshear bond strength among groups of BL, BLSA1, BLSA2 and BLSA3 (p>0.05).

When the bond strengths between the two adhesives were compared, no significant differences were found among CONTROL/SB, CONTROL/SE and BLSA3/SB (p>0.05). Moreover, there were no significant differences of bond strength between SB and SE groups in the same condition of irrigation time except for the group treated with SA for 10 min.

Percentages of failure mode in SB and SE groups are presented in Figure 1 and Figure 2, respectively. According to statistical analysis, there were no significant differences of the percentages of failure modes among the experimental groups in both type of adhesive. The adhesive failures were prominent in all groups.

Scanning electron microscopy (SEM) micrographs of the resin-enamel interfaces of all experimental groups using Adper™ Single Bond 2 (SB groups) are demonstrated in Figure 3 and those using Clearfil SE Bond (SE groups) were in Figure 4. No differences of interface morphology were found among groups bonded with the same adhesive system. For the SB groups, the SEM micrographs revealed uniformity of resin tags which were wider, longer and deeper penetration than those found in the SE groups.

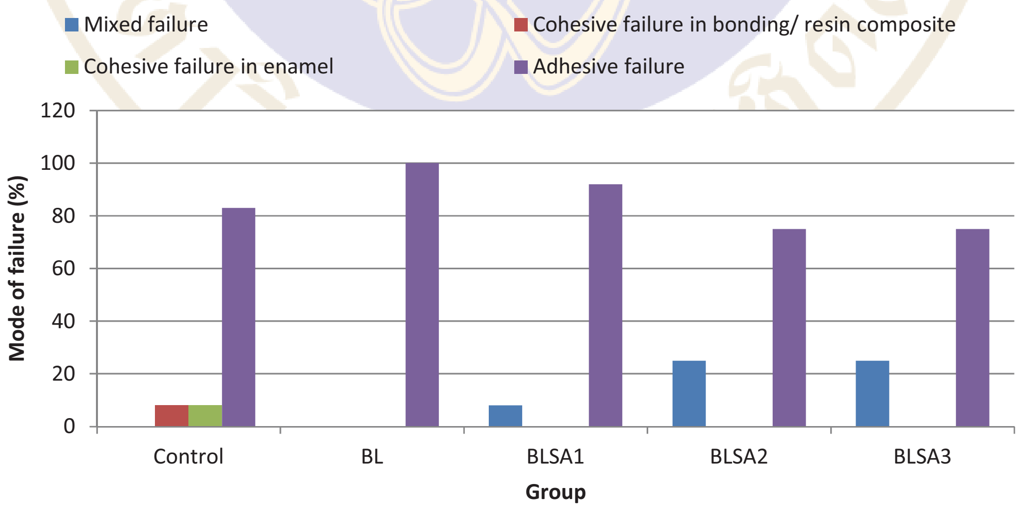

Figure 1 Percentage of failure modes in the specimens of Adper™ Single Bond 2 groups. BL: bleached group, BLSA1: bleaching and then irrigating with 10% sodium ascorbate for 30 s, BLSA2: bleaching and then irrigating with 10% sodium ascorbate for 1 min, BLSA3: bleaching and then irrigating with 10% sodium ascorbate for 10 min.

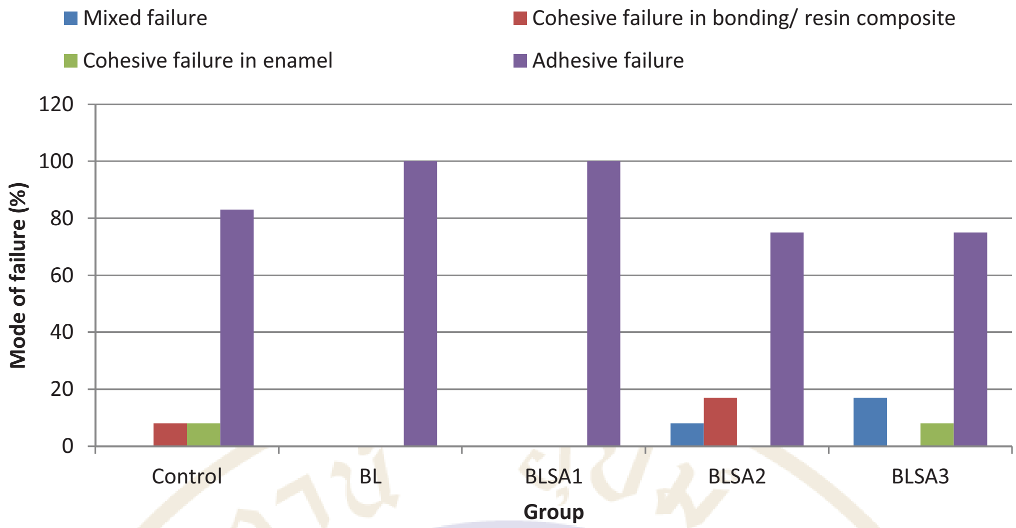

Figure 2 Percentage of failure modes in the specimens of Clearfil SE Bond groups. BL: bleached group, BLSA1: bleaching and then irrigating with 10% sodium ascorbate for 30 s, BLSA2: bleaching and then irrigating with 10% sodium ascorbate for 1 min, BLSA3: bleaching and then irrigating with 10% sodium ascorbate for 10 min.

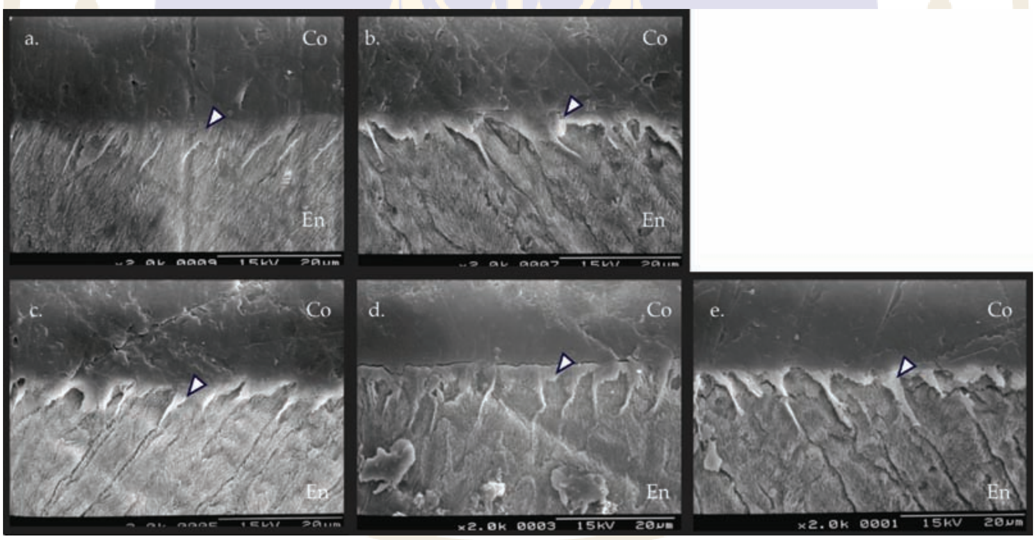

Figure 3 SEM images of the resin-enamel interface of Adper™ Single Bond 2 (SB) groups (x2000). Arrows indicate penetration of resin tags into enamel. Consistency uniform resin tags with well-defined appearances along the interfaces were observed. Interprismatic resin tags of approximately 10 microns length were revealed. No morphological differences of resin tag were found among experimental groups. Co: Resin composite, En: Enamel.

a. CONTROL/SB; unbleached group.
b. BL/SB, bleached group; bleached with 10% carbamide peroxide for 7 days.
c. BLSA1/SB; bleaching and then irrigating with 10% sodium ascorbate for 30 s.
d. BLSA2/SB; bleaching and then irrigating with 10% sodium ascorbate for 1 min.
e. BLSA3/SB; bleaching and then irrigating with 10% sodium ascorbate for 10 min.

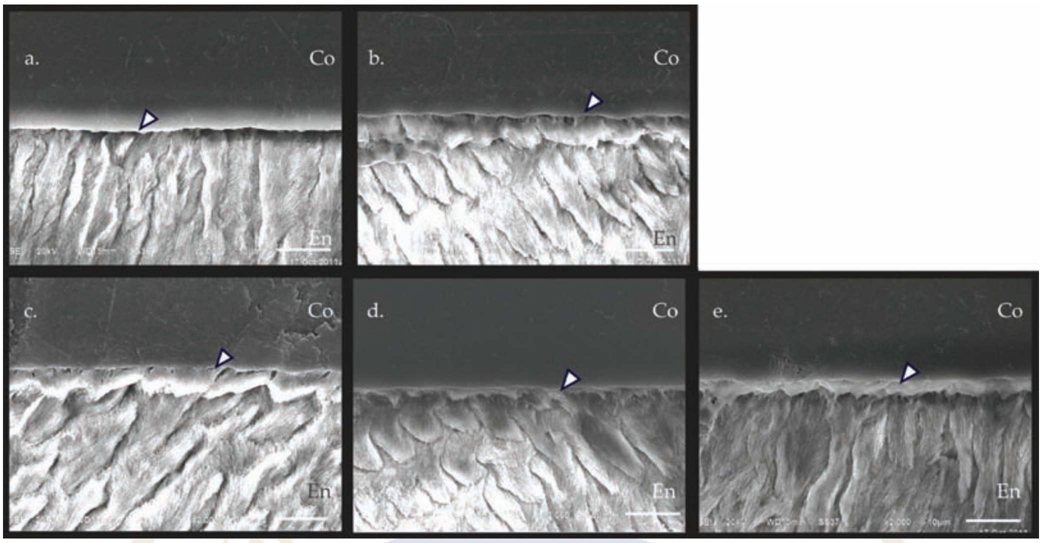

Figure 4 SEM images of the resin-enamel interface at of Clearfil SE Bond (SE) groups (x2000). Arrows indicate shallow penetration of resin into superficial surfaces of enamel. Well-defined interprismatic resin tags were not observed as in the SB groups. Morphological differences of resin penetration among the experimental groups were not found. The absences of resin infiltration in Figure 4a. and 4d. may be due to different tilting angles of the specimens. Co: Resin composite, En: Enamel.
a. CONTROL/SE; unbleached group.
b. BL/SE, bleached group; bleached with 10% carbamide peroxide for 7 days.
c. BLSA1/SE; bleaching and then irrigating with 10% sodium ascorbate for 30 s.
d. BLSA2/SE; bleaching and then irrigating with 10% sodium ascorbate for 1 min.
e. BLSA3/SE; bleaching and then irrigating with 10% sodium ascorbate for 10 min.

Discussion

This study was designed to used 10% carbamide peroxide for it was used in most of at-home tooth bleaching system. Most studies suggested that any adhesive restorations should be postponed for 1-3 weeks after bleaching since the bond strength is reduced if the procedure is performed immediately.[5-7,18-20] The results in this study indicated that the bleaching group which the bonding procedure was performed immediately after bleaching had statistically significant lower bond strength than the control group (unbleached). The results are in accordance with previous studies which reported that bond strengths of enamel decreased after bleaching treatment.[5-8] Bleaching agents affected the dental tissue[4,21-24] and also dental adhesive restorations.[5-8] Hydrogen peroxide and carbamide peroxide which commonly used as bleaching agents have strong oxidizing effect. Oxygen that released from carbamide peroxide during the bleaching process may inhibit polymerization of the adhesive. This resulted in lower bond strength for the bleached specimens[9,10] and might also affect the properties of material since the mechanical properties of polymer materials depend on their degree of polymerization.[18] In addition, the bleaching agent caused some changes in morphology of bleached enamel such as more porosity and loss of prismatic form.[25,26] These may be due to the effect of decreasing in calcium and phosphorous relative concentrations of the most superficial bleached enamel surface.[22] Then it turned to morphological change in crystallites of enamel.[27] Previous studies showed that the resin tags formation at resin-enamel interface observed from SEM in the unbleached group were more adjoining, contiguous and well defined than those found in the group that immediately applied adhesive after bleaching. Large areas of the enamel surface that free of resin were also detected in the bleached specimens.[28] However, the differences of the resin tag among the unbleached and bleached group bonded with the same adhesive system could not be detected in this study. This may be due to differences of type and concentration of bleaching agents. Moreover, the previous studies used the bovine enamel in their studies.

The results of this study showed that the BL group which the bonding procedure was performed immediately after bleaching had statistically significant lower bond strength than the control group (unbleached group) for both two adhesive systems. The results are in accordance with previous studies reported that bond strengths of enamel decreased after bleaching treatment in both etch-and-rinse and self-etch adhesive systems.[5,6] The results also revealed that the BL/SB group had statistically significant lower bond strength when compared to the BL/SE group. The differences of compromised bond strength in etch-and-rinse and self-etch adhesive systems are controversy.[5,6,8] This may depend on the materials and techniques used in the studies. The mechanism of bonding of etch-and-rinse adhesive system and self-etching system might explain the difference of bond strength to the bleached enamel. Clearfil SE Bond, self-etch adhesive, contains acidic monomer that has potential to etch and remove smear layer less than the etchant of Adper™ Single Bond 2.[29,30] Thus more smear layer are left on the enamel surface and these might conceal the residual oxygen that entrapped in the interprismatic layer of bleached enamel. Consequently, the inhibition of polymerization from these residual oxygen might be diminished. This brings to the higher bond strengths in BL/SE group. Additionally, Clearfil SE Bond has 10-methacryloxydecryl dihydrogen phosphate (10-MDP) as an acidic monomer that could have chemical bond to the calcium of hydroxyapatite of enamel surface.[30-32] These chemical bond could be another reason for the lesser compromised bond strength found in BL/SE compared to those of BL/SB group.

The application of some antioxidant agents such as sodium ascorbate[8,13,14] before applying the adhesive restoration can improve the compromised bond strengths.[33,34] The results of this study revealed that microshear bond strength of the BLSA3/SB group was higher than the bleached group and did not show any statistically significant differences from the control/SB group. Sodium ascorbate, sodium salt form of Vitamin C, has the antioxidant capacity to reduce oxidative effect of the residual peroxide from bleaching agent.[34-36] It allows free radical of the adhesive materials to proceed polymerization without interference. Therefore this could be responsible for the reversal of the compromised bond strength after applying the antioxidant onto bleached enamel. Even though sodium ascorbate solution had the reversal effect to the compromised bond strength of bleached enamel in the SB group that was treated with SA for 10 min (BLSA 3). However, the bond strengths of BLSA1/SB, BLSA2/SB groups, which application times of sodium ascorbate were 30 s and 1 min, were not different from the bleaching group, the bond strengths were slightly increase in these groups. It might need the extension of application time in the BLSA1/SB and BLSA2/SB group to improve bond strength of the resin composite on bleached enamel. Kaya and coworkers[37] studies the reversal of compromised bond strengths in 5 experimental groups of bleached enamel using different application periods; 10 min, 60 min, 120 min, 240 min and 480 min, of sodium ascorbate which used as an antioxidant prior to perform adhesive procedures. The result revealed that the bond strength of 10-min application group was statistically significant lower than those of 120-min, 240-min and 480-min groups. However, the bond strength of the 10-min and 60-min application groups were not significant difference from the unbleached group. It demonstrated that the application time affected the efficiency of antioxidant in reducing the oxidative effect of bleaching agent. This might infer that the longer contact time of sodium ascorbate to the bleached surface, the more residual oxygen was diminished. The results of present study are in agree with Kaya et al[37] that the 10-min SA application can reverse the compromised bond strength of bleached enamel.

For the groups using SE, sodium ascorbate was ineffective to reverse the bond strengths of bleached group. No statistically significant differences of the bond strength data among the antioxidant treatment groups and the BL group were found. Besides, bond strengths of these groups were lower than the control/SE group. When the bond strength of the two adhesives on the SA-treated groups were evaluated, the results indicated that the bond strength of the 10-min application of 10 % SA in SE group was lower than that of SB group. The null hypothesis of this study was rejected. The reasons for the differences in bond strength might be related with the different chemical compositions of the two adhesive systems.[38] Additionally, residual oxygen from bleaching process that affected resin polymerization mostly found on superficial enamel[10] could be reduced by the antioxidant effect of 10% sodium ascorbate and let the adhesives infiltrate and polymerize without interference.[35,36] Sodium ascorbate is a sodium salt of ascorbic acid which can form stable complexes with calcium ions.[39] Once the Clearfil SE Bond, a mild two-step self-etching adhesive contains 10-MDP as an acidic monomer which can chemically bond to calcium of hydroxyapatite[40] was applied, ascorbate ions might probably scavenge calcium ions on the enamel surface.[41] So less calcium is available for insoluble salt formation with the 10-MDP functional monomer of SE, hence the bond strengths of BLSA3/SE group could not be reversed in the same level as in SB group. Further researches should be performed to clarify the effect of sodium ascorbate on the durability of the resin-enamel bonds and to evaluate the effect of other antioxidants for a more effectiveness in a short application period.

Within the limitations of this study, it can be concluded that 10% of carbamide peroxide had the adverse effect on the microshear bond strengths of two adhesive systems on the bleached enamel.

Ten-minutes application of 10% sodium ascorbate could reverse the compromised bond strength in Adper™ Single Bond 2.

Ten percent of sodium ascorbate could not reverse the compromised bond strength in Clearfil SE Bond.

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