

# Effects of applying antioxidants on bond strength of bleached bovine dentin

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**Objectives:** Some antioxidants are believed to restore dentin bond strength after dental bleaching. This study was done to evaluate the influence of antioxidants on the bond strength of bleached bovine dentin. **Materials and Methods:** Thirty incisors were randomly assigned to 10 groups (two unbleached control and eight bleached groups: immediate bonding IB, 4 wk delayed bonding DB, 10% sodium ascorbate treated SA, 10%  $\alpha$ -tocopherol treated TP groups). Teeth in half of groups were subjected to thermal stress, whereas the remaining groups were not. Resin-dentin rods with a cross-sectional area of 2.25 mm<sup>2</sup> were obtained and microtensile bond strength was determined at a crosshead speed of 1 mm/min. Fifteen specimens were prepared for SEM to compare the surface characteristics of each group. The change in dentin bond strength from thermal stress and antioxidant treatment was evaluated using two-way analysis of variance (ANOVA) and Sheffe's *post hoc* test at a significance level of 95%. **Results:** The control group exhibited the highest bond strength values, whereas IB group showed the lowest value before and after thermocycling. The DB group recovered its bond strength similar to that of the control group. The SA and TP groups exhibited similar bond strength values with those of the control and DB groups before thermocycling. However, The TP group did not maintain bond strength with thermal stress, whereas the SA group did. **Conclusions:** Applying a 10% sodium ascorbate solution rather than 10%  $\alpha$ -tocopherol solution for 60 sec is recommended to maintain dentin bond strength when restoring non-vital bleached teeth. (*Restor Dent Endod* 2015;40(1):37-43)

**Key words:** Antioxidant; Carbamide peroxide; Microtensile bond; Sodium ascorbate strength; Tooth bleaching;  $\alpha$ -tocopherol

## Introduction

Dental bleaching has become a very popular treatment. Despite excellent esthetic outcomes, many studies have shown that dental bleaching reduces enamel bond strength of the composite from bovine and human teeth when bonding is performed immediately after bleaching.<sup>1-4</sup> This reduction in bond strength is due to residual oxygen left by the bleaching agent, which inhibits polymerization of resin-based materials.<sup>3,4</sup> Other causes reported are morphological, physical, and chemical alterations of the dental hard tissues.<sup>5-7</sup>

After the dental substrate has been exposed to bleaching agents, a certain period of time must elapse for the restorative procedure to be performed effectively. The time

for bonding procedures varies from 24 hours to 3 weeks.<sup>3,8</sup> If the bleaching procedure is immediately followed by adhesive restoration, the adhesive interface may be damaged, resulting in leakage or fewer, shorter, and poorly shaped resin tags in the dental enamel.<sup>4,9</sup>

Sodium ascorbate (SA) solution is a neutral, biocompatible antioxidant.<sup>4</sup> In studies by Lai *et al.*, decreased enamel bond strength after bleaching was returned to normal when bleaching was followed by SA treatment.<sup>4,7</sup> Other studies have demonstrated that applying 10% SA to enamel and dentin after bleaching reverses bond strength loss.<sup>10-12</sup>

Another antioxidant,  $\alpha$ -tocopherol, may be a candidate bond strengthening agent when used immediately after dental bleaching. However, few studies have assessed vitamin E or  $\alpha$ -tocopherol as an anti-oxidizing agent.<sup>13</sup>  $\alpha$ -Tocopherol is the most active component of the vitamin E complex, and is a powerful antioxidant in the human body in the lipid phase.<sup>14</sup> The critical role of  $\alpha$ -tocopherol protecting against free-radical reactions becomes apparent when considering the vast number of diseases and conditions, such as aging, many types of cancer, atherosclerosis and other circulatory diseases, arthritis, cataract formation, senile dementia (Alzheimer's type) and

respiratory diseases induced by pollution that are thought to be caused by these reactions.<sup>15</sup>

The aim of this study was to evaluate the influence of antioxidants (SA and  $\alpha$ -tocopherol) after non-vital bleaching on the microtensile bond strength of bovine dentin when used immediately after dental bleaching. The hypotheses in this study were that immediate dentin bonding after dental bleaching reduces bond strength and use of antioxidants restores lost bond strength.

## Materials and Methods

Thirty bovine incisors were used in this study. The teeth were randomly assigned to 10 groups (two unbleached control groups and eight experimental bleached groups) and were stored in distilled water ( $37 \pm 1^\circ\text{C}$ ). The experimental groups were divided according to the factors of delayed time for bonding after bleaching and use of antioxidants. Teeth in groups 6 - 10 were subjected to thermal stress, whereas teeth in the remaining groups were not (Table 1). The composition, manufacturers, and batch numbers of the materials used in this study are presented in Table 2.

**Table 1.** Experimental design

Code	Group treatment	No-thermocycling	Thermocycling
Control	bonding without bleaching	Group 1	Group 6
IB	immediate resin bonding after bleaching	Group 2	Group 7
DB	delayed resin bonding 4 wk after bleaching	Group 3	Group 8
SA	10% sodium ascorbate solution right after bleaching	Group 4	Group 9
TP	10% $\alpha$ -tocopherol solution right after bleaching	Group 5	Group 10

IB, immediate bonding group; DB, delayed bonding group; SA, 10% sodium ascorbate group; TP, 10%  $\alpha$ -tocopherol group.

**Table 2.** Materials used in this study

Materials	Composition	Manufacturers
Opalescence PF 15%	15% carbamide peroxide gel, potassium nitrate, 0.11% fluoride ion	Ultradent Products, South Jordan, UT, USA
Adper Single Bond 2	Bis-GMA, HEMA, dimethacrylate, methacrylate functional copolymer of polyacrylic and polytaconic acid, water, alcohol, photoinitiator	3M ESPE, St. Paul, MN, USA
Filtek Z350	Bis-GMA, UDMA, TEGDMA, Bis-EMA, 20 nm nanosilica and zirconia particles	3M ESPE, St. Paul, MN, USA
Scotchbond Etchant Gel	37% phosphoric acid	3M ESPE, St. Paul, MN, USA
Sodium ascorbate	$\text{C}_6\text{H}_7\text{NaO}_6$ (powder)	Sigma-Aldrich, St. Louis, MO, USA
$\alpha$ -Tocopherol	$\text{C}_{29}\text{H}_{50}\text{O}_2$ (solution)	Sigma-Aldrich, St. Louis, MO, USA

Bis-GMA, Bisphenol A glycidyl methacrylate; HEMA, Hydroxyethylene  $\alpha$  methacrylate; UDMA, Urethane dimethacrylate; TEGDMA, Triethylene glycol-dimethacrylate; Bis-EMA, Bisphenol A ethoxylate dimethacrylate.

Thirty freshly extracted lower bovine incisors were selected. The teeth were scraped to clean any residual tissues, and were washed under running tap water immediately after extraction. The root was cut about 5 mm below the cemento-enamel junction for convenience. The pulp chamber was accessed from the lingual surface using a #330 carbide bur and Endo Z bur in a high-speed hand-piece with water cooling. The pulp was removed with barbed broaches, and the endodontic cavity was finalized with a #3 low speed round bur irrigated with distilled water. The cavity base was filled with glass ionomer cement.

Control groups 1 and 6 remained unbleached, whereas the others were bleached. In the bleached groups, 15% carbamide peroxide gel (Opalescence, Ultradent, South Jordan, UT, USA) was applied in the access cavity 6 hours per day for 7 days according to the manufacturer's instructions. At the end of the daily bleaching procedure, the teeth were rinsed with air-water spray for 60 seconds and air dried. Then, the bovine teeth were immersed in distilled water at 37°C until the next bleaching gel application. In the delayed bonding groups 3 and 8, the cavities were washed with distilled water and dried with compressed air after 7 days of bleaching treatment and then stored in distilled water at 37°C for 4 weeks.

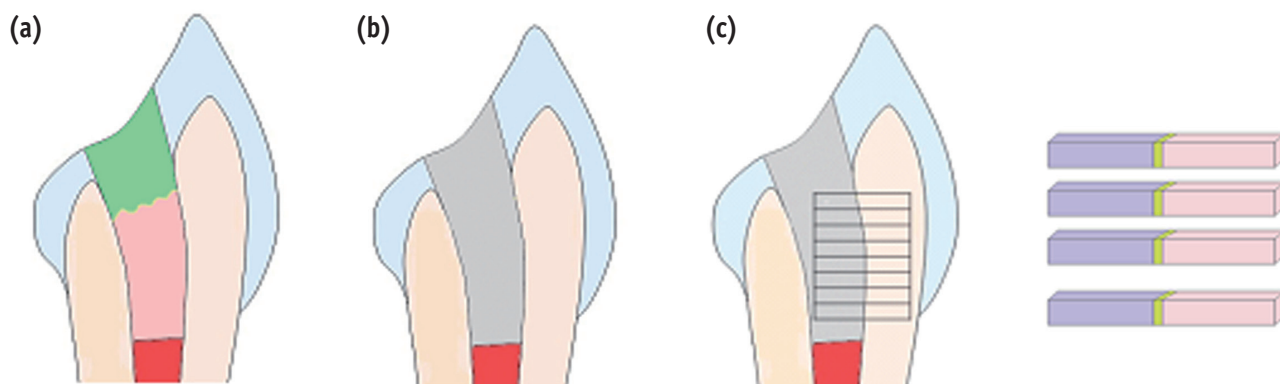
SA was dissolved in distilled water and  $\alpha$ -tocopherol was dissolved in ethanol to make 10% solutions. The 10% SA solution was applied into the accessed cavity for 60 seconds. After the antioxidant treatments, the cavities were thoroughly rinsed with distilled water for 30 seconds and gently dried. The 10%  $\alpha$ -tocopherol solution was applied in the same way.

The cavities were etched with 37% phosphoric acid (Scotchbond Etchant, 3M ESPE, St Paul, MN, USA) for 15

seconds and washed with air-water spray for 30 seconds. Excess water was removed with a cotton pellet, leaving the substrate surface moist. Subsequently, Adper Single Bond 2 (3M ESPE) was applied to the prepared enamel and dentin surfaces according to the manufacturer's instructions, and light activated for 10 seconds with a curing unit (Curing Light XL 3000, 3M ESPE). The cavities were filled with Filtek Z350 composite resin (3M ESPE), which was inserted in multiple increments of 2 mm thickness each and light cured. The teeth in the thermocycled groups were subjected to 10,000 thermal cycles between 5 and 55°C, with a dwell time of 15 seconds.

### Microtensile bond strength test

The restored specimens were stored in 37°C distilled water for 24 hours. Each specimen was sectioned in a labio-lingual direction to provide two sections of 1.5 mm thickness each. The specimens were serially sectioned in an axial direction into 1.5 mm thick dentin-resin slabs and then rotated 90° and sectioned again to obtain resin-dentin rods from the pulp chamber wall with a rectangular cross-sectional area of approximately 2.25 mm<sup>2</sup> using an RB205 diamond cutter Metsaw-LS (R&B Inc., Daejeon, Korea) (Figure 1). Specimens with 10 rods in each group were selected. Each bonded surface area was calculated before testing by measuring the narrowest portion with a digital caliper (Mitutoyo, Kawasaki, Japan). The rods were attached to a testing apparatus (Microtensile tester, BISCO, Schaumburg, IL, USA) with cyanoacrylate adhesive (Zapit, DVA, Corona, CA, USA) applied to the composite and dentin sides of the rods. After setting, they were subjected to tensile forces at a crosshead speed of 1 mm/min. Microtensile bond strength was determined in MPa.



**Figure 1.** Specimen preparation for microtensile bond strength test. (a) Access cavity preparation and non-vital bleaching; (b) Bonding substrate were restored with adhesive and composite to cavity wall; (c) Resin-dentin rods with a rectangular cross-sectional area of approximately 1.5 × 1.5 mm.

### Scanning electron microscopy (SEM)

Fifteen dentin specimens, which were surface treated in the same way for the true bond strength test, were prepared and divided into five groups. The samples were washed in distilled water and air-dried before being prepared for SEM. The specimens were sputter coated with platinum. Photomicrographs were obtained from randomly chosen areas on the surfaces at a magnification of  $\times 700$ . (S-3000N/H, Hitachi High-Technologies, Tokyo, Japan)

### Statistical analysis

The antioxidant data were analyzed by two-way analysis of variance (ANOVA), one-way ANOVA, and Sheffe's *post hoc* test. The change in dentin bond strength from thermal stress was evaluated using the independent sample *t*-test. The statistical analysis was carried out with the SPSS 19.0 software system (SPSS Inc., Chicago, IL, USA). A *p* value  $< 0.05$  was considered significant.

## Results

### Microtensile bond strength test

The results of the microtensile bond strength test are presented in Table 3. Results of a two-way ANOVA indicated significant differences for 'treatment modality' ( $p < 0.001$ ) and the 'thermocycling' ( $p < 0.001$ ) factors and for the interaction between the factors ( $p = 0.036$ ). The unbleached control group exhibited the highest bond strength values before and after thermocycling. Group IB, immediately bonded after bleaching, showed the lowest bond strength value without thermocycling ( $p < 0.05$ ) and its value further decreased following thermocycling process ( $p < 0.01$ ). However, the 4 week delayed bonding DB group recovered its bond strength similar to that of the control group with and without thermocycling.

Among the groups treated with antioxidants, the SA group exhibited similar bond strength values with those of

the control and DB groups, and no difference was observed following the thermocycling treatment. The TP group exhibited a similar strength value compared to that of the SA group without thermocycling, whereas thermocycling brought about decreased bond strength, although it was greater than that of the IB group ( $p < 0.05$ ).

Approximately 10,000 cycles of thermal stress lowered bond strength except in the control, SA, and DB groups (*t*-test). The lowest bond strength value before thermocycling was revealed in the IB group. No significant differences were found among the remaining groups. The lowest bond strength value after thermocycling was in the IB group followed by the TP group. No significant differences were observed among the remaining groups.

### SEM

The dentinal tubules were relatively well exposed without a smear layer in the control group (Figure 2a). An etched pattern without exposure of the dentinal tubules was found on the surface of the dentin, bleached with 15% carbamide peroxide gel in the IB group (Figure 2b). Sparsely closed dentinal tubules were observed on the surface of the 10% SA solution group (Figure 2c). The surface bleached with 10%  $\alpha$ -tocopherol solution showed an etched pattern with fewer dentinal tubules exposed than those in the SA group (Figure 2d). A comparatively clean surface was observed in the DB group after storage in distilled water for 4 weeks, with sparsely opened dentinal tubules (Figure 2e).

## Discussion

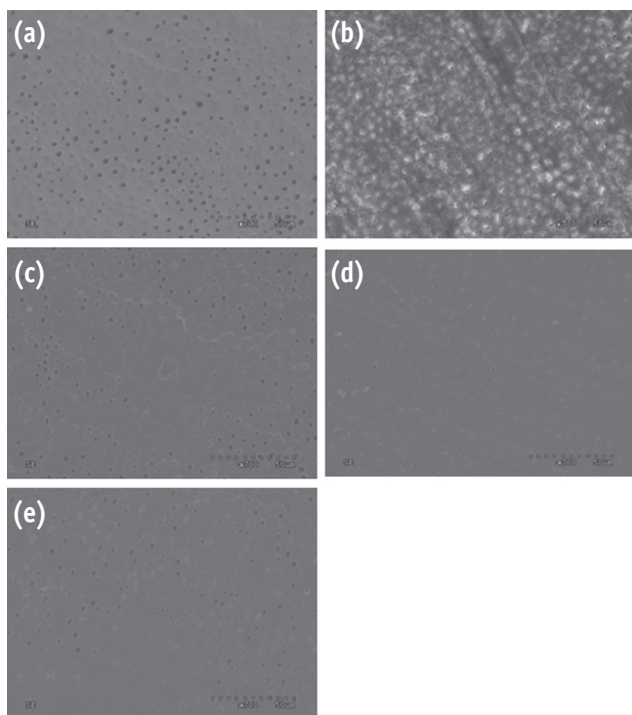
No significant differences in bond strength or microleakage behavior are found between bovine and human teeth.<sup>16,17</sup> Camargo *et al.*, concluded that dentin exposed at the incisal surface of human and bovine teeth presents similar clinical and micro-morphological aspects, as represented by surfaces with equivalent numbers of open dentinal tubules.<sup>18</sup> Additionally, the coronal dentin layers of human deciduous and permanent molars as well as those of bovine

**Table 3.** Means and standard deviations of microtensile bond strength test values (MPa)

Group	No-thermocycling	Thermocycling	Results of Two-way ANOVA
Control (Group 1, 6)	21.93 $\pm$ 4.14 <sup>a</sup>	20.20 $\pm$ 3.46 <sup>a</sup>	Main effects
IB (Group 2, 7)	15.49 $\pm$ 3.49 <sup>b</sup>	10.75 $\pm$ 2.10 <sup>c</sup>	Treatment modalities $p < 0.001$
DB (Group 3, 8)	21.40 $\pm$ 3.49 <sup>a</sup>	20.59 $\pm$ 2.95 <sup>a</sup>	Thermocycling $p < 0.001$
SA (Group 4, 9)	21.95 $\pm$ 3.78 <sup>a</sup>	19.83 $\pm$ 3.06 <sup>a</sup>	Interaction effect
TP (Group 5, 10)	19.43 $\pm$ 3.23 <sup>a</sup>	15.89 $\pm$ 2.26 <sup>b</sup>	Treatment modalities x Thermocycling $p = 0.036$

Different letters indicate the statistically significant difference at  $p < 0.05$ .

IB, immediate bonding; DB, delayed bonding; SA, 10% sodium ascorbate; TP, 10%  $\alpha$ -tocopherol.



**Figure 2.** SEM photograph showing dentin surface treated with bleaching agent, antioxidants and without thermocycling. (a) Control group; (b) Immediate group; (c) 10% sodium ascorbate group; (d) 10%  $\alpha$ -tocopherol group; (e) After 4 weeks group.

central incisors are not significantly different in the number of tubules/mm<sup>2</sup> or tubule diameter. These results suggest that bovine dentin is a suitable substitute for human molar dentin in adhesion studies when standardized preparations are used.<sup>19</sup>

Opalescence PF 15% gel (Ultradent), which contains 15% carbamide peroxide, potassium nitrate, and 0.11% fluoride was used as the non-vital bleaching agent. It was injected into the pulp cavity and came in direct contact with the dentin. Bond strength of composite resins was lower on dentin when immediately bonded just after bleaching.

The bleached specimens in the IB groups without any antioxidant treatment (Groups 2 and 7) showed the lowest bond strength values with bond failure at the interface between the tooth substrate and the bonding agent. This was probably due to residual oxygen on the tooth surface inhibiting polymerization of the bonding agent. As a result, the oxygen-rich tooth structure did not provide a good surface for bonding. As reported by Attin *et al.*, the effect of the bleaching agent reached the inside of the tooth structure.<sup>20</sup> Opalescence Xtra, which is a 35% peroxide-based bleaching agent, produces a much more irregular

pattern on the dentin, with shallow erosive areas covering the sample surface.<sup>6</sup> However, when the SA solution was applied to the tooth surface, the bond strength of the adhesive to the bleached tooth surface was maintained at a level equivalent to that of non-bleached surfaces in this study. This result suggests that the cause of reduced bond strength was due to oxygen ions produced by the bleaching agent.

SA is a sodium salt of ascorbic acid and a well-known antioxidant. SA is capable of reducing a variety of oxidative compounds, particularly free radicals.<sup>21</sup> One study demonstrated the potential protective effect of SA against hydrogen peroxide-induced damage in biological systems.<sup>22</sup> Ascorbic acid also shows high antioxidant activity. However, its pH is approximately 1.8, which makes it inappropriate for clinical use. In contrast, SA has a pH of 7.4, but its antioxidant activity is similar to that of ascorbic acid.<sup>23</sup>

The antioxidizing ability of SA helped to neutralize and reverse the oxidizing effects of the bleaching agent. Therefore, the altered re-dox potential of the oxidized bonding substrate is restored and polymerization of the adhesive continues without permanent termination.<sup>4</sup> The results of our study support these findings, as no significant difference was found between the DB and SA groups when compared with the unbleached control groups. Furthermore, we believe this bond-strength maintaining effect by SA is stable because it was observed despite 10,000 thermal stress cycles between 5 and 55°C.

Vitamin C, E, and vitamin precursors (e.g., carotenoids), which reduce the rate of formation or prevent propagation of free radicals, are notable non-enzymatic antioxidants. Of the vitamins, vitamin E is particularly important in preventing lipid peroxidation, whereas vitamin C reacts effectively with superoxide and hydroxyl radicals.<sup>24</sup> Vitamin E is the term used for a group of tocopherols and tocotrienols, of which  $\alpha$ -tocopherol has the highest biological activity. Vitamin E functions as a chain-breaking antioxidant that prevents propagation of free radical reactions.<sup>25</sup> It has been used on dentin and enamel with good bonding results and shows antioxidant activity similar to that of ascorbic acid.<sup>23,26</sup> As shown by Buettner, vitamins C and E are water and lipid soluble small antioxidant molecules that cooperate to protect lipid membranes against free radical processes in organisms.<sup>27</sup> Furthermore, vitamin E is more oxidizing and stable than ascorbate because of its hydrophobicity.<sup>23</sup>

However, the presence of alcohol in the vitamin E composition formulated for this study may have contributed to the good response in terms of antioxidant activity, as vitamin E is not usually miscible in water solutions.<sup>26</sup>  $\alpha$ -Tocopherol formulated in this solution resulted in a significant increase in bonding strength of the bleached enamel. A previous study showed that the use of adhesive

systems containing organic solvents such as ethanol or acetone reverses the negative effects of bleaching on bonding.<sup>26</sup> It has also been observed that applying alcohol on bleached enamel increases bond strength, although the values do not return to the levels of a non-bleached group.<sup>28</sup> This restrictive increase may be related to the fact that bleached enamel is more porous and, therefore, has more water-containing oxygen.<sup>29</sup> We observed similar results in this study; that is, the TP group like the SA group exhibited a bond strength-maintaining effect in the bleached specimens only; however, thermal stress reduced this bond strength. Another surface morphology factor may be related with the reduced bond strength. It was inferred from the SEM image of the TP treated dentinal surface that fewer dentinal tubules were exposed than those on the SA treated surface.

Further studies should be conducted to evaluate the antioxidant potential of sodium ascorbate and  $\alpha$ -tocopherol to determine stability of the antioxidant activity, and the proper concentration and time for a more valuable effect on dentin.

## Conclusions

We conclude that dentin bond strength was significantly reduced when bonding was performed immediately after bleaching using 15% carbamide peroxide bleaching gel. A 4 week wait period between bleaching and composite resin bonding significantly restored bond strength with the non-vital bleaching technique. Surface treatment with 10% SA and  $\alpha$ -tocopherol for 60 seconds resulted in almost the same bond strength as that of the control and 4 week groups without thermal stress. However, the  $\alpha$ -tocopherol group did not maintain bond strength with thermal stress, whereas the SA group did. Therefore, applying SA for 60 seconds is recommended if dentin bond strength is to be maintained when restoring non-vitally bleached teeth.

Conflict of Interest: No potential conflict of interest relevant to this article was reported.

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