Improvement of Bonding to Bleached Bovine Tooth Surfaces by Ascorbic Acid Treatment

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The purposes of this study were twofold: (1) to examine the effects of bleaching on the bond strength of an adhesive to bovine tooth surfaces; and (2) to explore the effectiveness of an ascorbic acid application in preventing the deterioration of bonding ability due to bleaching. In the experimental groups, ascorbic acid was applied to the bleached bovine tooth surfaces. In the control groups, the tooth specimens were bleached but no ascorbic acid application was carried out. All bonded specimens were subjected to shear bond test, and the data were statistically analyzed with two-way ANOVA and Bonferroni’s test (p=0.05). Bond strengths to the bleached specimens were significantly lower than those of non-bleached specimens. No statistical differences were found in bond strength between the bleached and non-bleached groups when the ascorbic acid treatment was carried out. Results of this study suggested that ascorbic acid application was effective in preventing the reduction of bonding ability to bleached teeth.

Keywords: Bleaching, Shear bond strength, Ascorbic acid

INTRODUCTION

Presently, two tooth bleaching methods are available: at-home bleaching and in-office bleaching¹². To date, many studies have reported on the bond strength of composite resins with adhesives on bleached tooth surfaces²⁰. For composite resins that were bonded to tooth surfaces immediately after bleaching, their shear bond strengths were significantly lower than those of non-bleached teeth²⁰. Furthermore, it has been reported that the weakening of the bond occurred both superficially and internally¹⁰,¹¹.

Hydrogen peroxide, urea peroxide, or sodium perborate may be used alone or in combination as bleaching agents as they provide oxidative effects against stain-causing substances¹⁹. Although the bleaching process and speed differ according to the type or combination of chemicals used, the oxygen released from the chemicals plays the main role in the reaction. It is believed that residual oxygen remains on the tooth surface or inside the tooth after the bleaching treatment. Consequently, this inhibits the polymerization of resin monomers, thereby causing reduced bond strength of the restorations¹³-¹⁶. However, with the passage of time, the residual oxygen slowly dissipates so that oxidation is reduced. In this manner, resins are able to achieve their intended and inherent bond strengths if the bleached surface were restored after a certain time interval¹⁷-²⁰. Therefore, it is recommended that composite restorations be carried out one or two weeks after bleaching²,¹⁶,²¹-²⁴. It is preferable, however, to close root canals or perform restorative treatment with resin materials immediately after bleaching to prevent infection or recurrent discoloration, or to complete esthetic restorations immediately. To achieve this, resin materials need to provide the same bond strength to bleached tooth surfaces as to non-bleached surfaces.

Studies conducted by Lai et al.²⁵,²⁶ have shown that hydrogen peroxide- or sodium hypochlorite-induced reduction in bond strength of composite resin to dentin and enamel was reversed with the use of an antioxidant, sodium ascorbate. If antioxidant treatment of bleached enamel before bonding could reverse the reduced bond strength of composite resin, it may thus be a viable alternative to the delayed restorative procedure after bleaching. The purpose of this study was to improve the bond strength of an adhesive to both ground and non-ground bleached bovine tooth surfaces by using ascorbic acid as a reducing agent²⁷. The hypothesis was that the application of ascorbic acid to bleached surfaces provides a bond strength that is equivalent to that of non-bleached surfaces.

MATERIALS AND METHODS

Materials

Materials used in this study are presented in Table 1. Bleaching agent used was a mixture of 30 wt% hydrogen peroxide and sodium perborate tetrahydrate powder (ratio by weight of liquid to powder was 1:1). Bleaching time was set to one week, as per the common routine period for walking bleach technique.

An ascorbic acid solution of 10 wt% was used as a surface treatment agent to eliminate residual oxygen produced by the bleaching agent. Through preliminary experiment, it was found that the physical properties of bovine teeth were weakened with long-
Improvement of bonding to bleached bovine tooth

Table 1  Materials used in this study

<table>
<thead>
<tr>
<th>Material</th>
<th>Lot Number</th>
<th>Manufacturer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrogen Peroxide (30 wt%)</td>
<td>M4P2818</td>
<td>Nacalai Tesque Inc.</td>
</tr>
<tr>
<td>Sodium Perborate Tetrahydrate (Powder)</td>
<td>V5B4987</td>
<td>Nacalai Tesque Inc.</td>
</tr>
<tr>
<td>Ascorbic Acid (10 wt%)</td>
<td>M8E8026</td>
<td>Nacalai Tesque Inc.</td>
</tr>
<tr>
<td>Clearfil SE Bond Primer</td>
<td>00488A</td>
<td>Kuraray Medical</td>
</tr>
<tr>
<td>Clearfil SE Bond Bond</td>
<td>00727A</td>
<td>Kuraray Medical</td>
</tr>
<tr>
<td>Clearfil Flow FX</td>
<td>0004AA</td>
<td>Kuraray Medical</td>
</tr>
</tbody>
</table>

![Specimen preparation procedure and classification of surface treatments.](image)

Fig. 1  Specimen preparation procedure and classification of surface treatments.

time application of ascorbic acid. In light of this finding, application time of ascorbic acid was set to one minute.

To fabricate bond test specimens, a bonding agent (SE Bond, Kuraray Medical) and a composite resin (Clearfil Flow FX, Kuraray Medical) were used.

**Tooth specimens**

Enamel and dentin surfaces of anterior bovine teeth were used as adherends. After removal of soft tissue and rinsing with distilled water, the teeth were stored at 4°C in a saline containing thymol until use. The labial surfaces of the crowns of bovine teeth were trimmed to fabricate 100 adherend surfaces, i.e., 50 enamel and 50 dentin surfaces (Fig. 1). Both enamel and dentin surfaces were ground flat using 600-grit SiC paper under water irrigation.

**Surface treatment**

Fig. 1 gives an overview of the surface treatment procedures in this study. For the bleaching treatment, 40 each of enamel and dentin adherend surfaces were immersed in the bleaching agent at 37°C for a week. After which, bleached adherends were thoroughly rinsed with distilled water to remove the bleaching agent from the surface. For the control specimens, 10 each of enamel and dentin adherends were used without the bleaching procedure (E-Cont, D-Cont).

For the ascorbic acid treatment, a 10 wt% ascorbic acid solution was applied using a sponge pellet to bleached enamel and dentin adherends (10 each) and left for one minute. The specimens were then rinsed with distilled water and dried with compressed air (E-BA, D-BA). For bond test specimens without the ascorbic acid treatment (non-treated group), 10 each of bleached enamel and dentin adherends were used (E-B, D-B).

To evaluate the effect of the bleaching agent on the inside of tooth structure, 20 each of bleached enamel and dentin adherends were ground down 0.5 mm on the bonding side using 600-grit SiC paper under water irrigation. 10 each of enamel and dentin adherents were treated with 10 wt% ascorbic acid...
in the same manner as for the non-ground bleached adherends (E-BGA, D-BGA). The remaining 10 each of enamel and dentin adherents were used as specimens without ascorbic acid treatment (E-BG, D-BG).

**Shear bond test**

The surface of each adherent, which had been subjected to any of the surface treatments as described above, was first pretreated with SE Bond primer for 20 seconds and dried with compressed air. Then, the bonding agent of SE Bond was applied and photo-polymerized for 30 seconds by a Light II (GC, Tokyo, Japan). To delimit the bonding area, it was done by placing a piece of masking tape with a 5.0-mm diameter opening. A brass ring (6.0 mm internal diameter, 2.0 mm height) was affixed, and Clearfil Flow FX was filled into the ring and photo-polymerized for three minutes. These specimens were stored in 37°C distilled water for 24 hours before they were subjected to a shear bond test.

Shear bond test was conducted using a universal testing machine (AutoGraph, AGS-5kNG, Shimadzu) at a crosshead speed of 1.0 mm/minute (Fig. 2). Failure patterns of the specimens were classified by an optical scanning microscope (SMZ-10, Nikon, Japan) with ×50 magnification. Each specimen comprised three distinct layers—namely resin layer, bonding layer, and tooth layer. Data were statistically analyzed by two-way ANOVA. Significant differences among groups with the two factors of grinding and ascorbic acid application were determined by Bonferroni’s multiple comparison test at 95% confidence level.

**SEM observation**

The surfaces of specimens with surface treatments were observed by a scanning electron microscope (SEM) (JSM-1100, JEOL, Tokyo, Japan). Specimens for SEM observation were prepared by making replicas of adherent surfaces using a polysiloxane impression material (Exafine, GC, Tokyo, Japan) and an epoxy resin (Epofix, Struers, Toyo, Japan). The specimens were then coated with gold by an ion sputtering device (Fine Coat Ion Sputter, JFC-1100, JEOL, Tokyo, Japan).

**RESULTS**

Fig. 3 and 4 present the results of shear bond strengths to enamel and dentin respectively. Both enamel and dentin specimens subjected to bleaching exhibited significantly lower values compared to those of non-bleached groups. Furthermore, the bond strength dropped even when the bleached layer was eliminated by grinding. There were no significant differences in bond strength between the ground and non-ground surfaces.

When ascorbic acid was applied for one minute, bond strength was remarkably improved on both non-

![Fig. 2 Schematic illustration of shear bond test.](image-url)

![Fig. 3 Shear bond strengths to enamel. Bars with the same letter indicate no significant differences. (p>0.05)](image-url)

![Fig. 4 Shear Bond strengths to Dentin. Bars with the same letter indicate no significant differences. (p>0.05)](image-url)
Improvement of bonding to bleached bovine tooth

Table 2 Failure pattern after shear bond test

<table>
<thead>
<tr>
<th></th>
<th>Resin Layer</th>
<th>Bonding Layer</th>
<th>Tooth (Enamel or Dentin) Layer</th>
</tr>
</thead>
<tbody>
<tr>
<td>E-Cont</td>
<td>0</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>E-B</td>
<td>0</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>E-BA</td>
<td>0</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>E-BG</td>
<td>0</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>E-BGA</td>
<td>0</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>D-Cont</td>
<td>0</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>D-B</td>
<td>0</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>D-BA</td>
<td>0</td>
<td>0</td>
<td>10</td>
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<tr>
<td>D-BG</td>
<td>0</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>D-BGA</td>
<td>0</td>
<td>0</td>
<td>10</td>
</tr>
</tbody>
</table>

Fig. 5 and 6 present the results of SEM observation. After ascorbic acid treatment, SEM photographs of the bleached surfaces exhibited almost the same
details as those before bleaching. Without ascorbic acid treatment, the enamel surfaces had scratches
due to grinding, while the dentin surfaces were covered with a smear layer with no recognizable open
dentinal tubules. By contrast, the enamel surfaces treated with ascorbic acid after bleaching exhibited
a relatively coarse texture, while the dentin surfaces showed exposed dentinal tubules without smear
layer.
DISCUSSION

In the present study, bond strength was evaluated using bovine teeth. This was chiefly because the use of bovine teeth is considered acceptable. No significant differences in bond strength\textsuperscript{39} and microleakage behavior\textsuperscript{39} were reportedly found between bovine and human teeth.

For the bleaching agent, a mixture of hydrogen peroxide and sodium perborate tetrahydrate was used. In clinical situations, this mixture is usually used for the walking bleach technique, whereby the bleaching agent is injected into the pulp cavity and sealed, so that it will act from the inside. With walking bleach technique, the agent injected into the pulp cavity usually comes in contact with the dentin only — it rarely makes direct contact with the enamel. Nonetheless, the bleaching agent will eventually make its way through the dentinal tubules to the enamel. Consequently, it is often reported that the bond strength of composite resins was lower not only on dentin but also on enamel. With regard to bond strength, this study sought out to investigate how bleaching adversely affected the bond strength of an adhesive to both dentin and enamel, as well as to investigate if the application of a reducing agent such as ascorbic acid would improve the bonding ability.

In this study, the resin material had significantly lower bond strengths to bleached enamel and dentin specimens than it did to non-bleached specimens. In addition, reduced bond strength was also observed when the tooth surface was ground down 0.5 mm after bleaching. As reported by Attin et al.\textsuperscript{11a}, the effect of the bleaching agent was found to reach the inside of the tooth structure. However, when an ascorbic acid 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, regardless of the surface grinding condition. This result seemed to indicate that the cause of reduced bond strength was due to the oxygen ions produced by the bleaching agent. On the other hand, there were reports that claimed that the cause of reduced bond strength of resin materials was not oxygen ions, but the softening of the tooth structure caused by bleaching\textsuperscript{36-39}. In this study, the specimens used for the bond strength test failed within the bonding agent layer, whether the tooth surface was bleached or not. This finding thus led us to assume that the cause of reduced bond strength was the reduced cohesive strength of the adhesive layer.

Without the ascorbic acid treatment, the bleached
specimens failed at the interface between the tooth structure and bonding agent during the shear bond test. This was probably due to the residual oxygen on the tooth surface inhibiting the polymerization of the bonding agent. Ascorbic acid and its salts are well-known antioxidants. They are capable of reducing a variety of oxidative compounds, especially free radicals\textsuperscript{33,34}. Previous studies have demonstrated the potential protective effect of ascorbic acid against hydrogen peroxide-induced damage in biological systems\textsuperscript{35,36}.

In the present study, the ascorbic acid-treated specimens showed a cohesive failure, hence indicating enhanced bond strength. Through this result, the oxidation reducing effect by the ascorbic acid treatment was validated. The hypothesis that the application of ascorbic acid to bleached surfaces provides adhesives with bond strengths equivalent to those of non-bleached surfaces was thus accepted.

An ascorbic acid solution of 10 wt\% has an etching potential as it possesses an acidity of approximately pH 2. Thus, the bond strength of the resin material was probably maintained by micromechanical retention to an etched surface. At this juncture, it must be clarified that bond strength was not properly maintained by etching the bleached surfaces with phosphoric acid or various types of self-etching primers. Therefore, the effectiveness of ascorbic acid solution in preventing the deterioration of adhesive bonding was most probably due to its reducing nature. It could thus be concluded that polymerization inhibition due to residual oxygen both on and inside the bleached tooth was one of the chief causes of reduced bond strength of adhesives to bleached surfaces.

It has been reported that the bleaching agent infiltrated and dispersed in both enamel and dentin to affect the hard tissue\textsuperscript{11}. In the present study, specimens with the bleached layer removed also exhibited weakened bonding, as did the bleached specimens without the surface layer removed. It is noteworthy that the surface properties and modes of failure of the former specimens were also the same as the latter specimens. This result seemed to indicate that oxygen produced by bleaching remained not only on the surface, but also inside the tooth structure.

The SEM photographs of bleached specimens with ascorbic acid treatment revealed the enamel surface being etched and the dentin surface free of a smear layer. This was probably due to the high acidity of 10\% ascorbic acid used in this study. In other words, ascorbic acid was effective in maintaining the bond strength of the resin material not only by reducing the residual oxygen, but also by etching the tooth surface.

In the present study, ascorbic acid was selected due to its reducing ability and safety in the oral cavity. However, it should be noted that ascorbic acid solution gradually oxidizes with the passage of time and becomes less reductive. It is difficult to preserve an ascorbic acid solution in its original condition for a long period of time. In view of this difficulty, due consideration and further investigation are indeed needed with regard to the storage method and shelf life of ascorbic acid solution for clinical use. In addition, there is a possibility that the bleaching effect might be impaired by ascorbic acid. If this were to occur, bleached-away stains will reappear if the concentration or working time of the ascorbic acid solution is not appropriate. In view of these practical concerns for clinical applications, further studies are needed to better understand these issues and address these concerns.

CONCLUSIONS

This study examined the effect of bleaching on tooth surfaces, as well as explored a method of preventing the weakening of bond strength of an adhesive to bleached tooth surfaces. Within the limitations of this study, the following conclusions were drawn:

1. Bond strength of the adhesive to enamel and dentin was reduced due to bleaching.
2. When a 10 wt\% ascorbic acid solution was applied to the bleached tooth surfaces for one minute, bond strengths of the adhesive to bleached enamel and dentin surfaces were maintained at a level equivalent to those of non-bleached surfaces.
3. An ascorbic acid solution of 10 wt\% exhibited the potential to etch enamel and dentin surfaces.

REFERENCES

6) Cavalli V, de Carvalho RM, Giannini M. Influence of carbamide peroxide-based bleaching agents on the