Evaluation of the Effect of Various Antioxidants on the Shear Bond Strength of the Composite Resin to the Bleached Enamel: An In Vitro study

Somya Jain¹ Neetu Jindal¹ Renu Agarwal¹ Ajisha Raju¹ Shallu Bansal²

¹Department of Conservative Dentistry and Endodontics, Surendera Dental College and Research Institute, Sri Ganganagar, Rajasthan, India
²Department of Oral and Maxillofacial Surgery, Swargiya Dadasaheb Kalmegh Smruti Dental College and Hospital, Nagpur, Maharashtra, India

Address for correspondence Somya Jain, MDS, Department of Conservative Dentistry and Endodontics, Surendera Dental College and Research Institute, Sri Ganganagar 335001, Rajasthan, India (e-mail: jainsomya.sunanda@gmail.com).


Abstract

Objective Various intrinsic and extrinsic stains cause discolored teeth, which is of great concern to patients, which can be treated by bleaching, enamel microabrasion, veneers, and crowns. However, bleaching leads to reduced bond strength if adhesive restorations are performed immediately. Thus, the application of antioxidant agents after bleaching has been recommended, which are proved to act as free radical scavengers, improving the bond strength.

Materials and Methods A total of 120 extracted human maxillary incisor teeth were taken. Using a slow-speed diamond saw and a water coolant spray, the roots of all the teeth were removed approximately 2 mm below the cementoenamel junction. With the labial surface facing upward, each sectioned sample was embedded in the acrylic resin. Then, the central portion of the embedded tooth was ground flattened with 600-grit silicon carbide paper such that the labial enamel surface becomes smooth and evenly flat.

All 120 samples were divided into two control groups (n = 20), i.e., positive control group (n = 10) and negative control group (n = 10) and five experimental groups (n = 100), such that each experimental group had 20 samples. The enamel surface in both the control groups and groups treated with antioxidants was thoroughly rinsed off with distilled water for 30 seconds and subjected to bonding procedure.

Result Significant differences were observed among the experimental groups (p < 0.05). The samples that were treated with 10% sodium ascorbate (group III) demonstrated significantly higher mean shear bond strength than the other experimental groups (p < 0.05).

Keywords ▶ antioxidants
▶ bleached enamel
▶ shear bond strength

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Introduction

Esthetic excellence is achieved by conjunction of various restorative treatments and vital bleaching procedures. When used alone, vital bleaching has proved to be a safe and conservative method but when used in conjunction with various restorative materials, its mechanism of action has shown deduction in the physical properties of various restorative materials.

Accreditation of the sparse, short, and poorly defined resin tags goes to the modified inorganic and organic composition of the bleached enamel surface after immediate treatment with peroxide-based bleaching agents. Thus, bond strength of resin to enamel accounts for the quality of these tags, their incidence, and their depth of penetration.1

Thus, the important complication following bleaching procedure is decreased bond strength of composite resin to bleached enamel.2

Various clinicians suggested a delay of 2 to 3 days following bleaching to perform all restorative procedures, which has further trammelled the immediate esthetic procedure. However, the use of various herbal and synthetic antioxidants to curb the effect of free radicals following bleaching procedure, enabling the restorative procedures to be performed immediately, has been proposed by several recent studies, as antioxidants have shown to have free radical scavenging ability, which can reverse the reduced bond strength of resin to bleached enamel.3

Thus, the aim of this study is an in vitro evaluation of the effect of various antioxidants on the shear bond strength of the composite resin to the bleached enamel.

Materials and Method

A total of 120 human, single-rooted, caries-free, maxillary incisors, extracted for periodontal reasons, were taken for the study. The inclusion criteria were as follows: teeth that were intact, noncarious, and freshly extracted due to periodontal disease or orthodontic purposes.

All the teeth were examined with the stereomicroscope under ×20 magnification to exclude the teeth with cracks, fracture lines, caries, developmental anomalies, and existing restorations. Also, the teeth with extrinsic and intrinsic discolorations were also excluded from the study.

Samples were stored in distilled water until mounting to prevent bacterial growth. The roots of all teeth were sectioned transversally 2 mm below the cementoenamel junction using a slow-speed diamond saw under a water coolant spray. Then, each sectioned coronal sample was embedded in the acrylic resin with the labial surface facing upward. Then, this mounted sample was subjected to bonding procedure. The composite resin was placed on the coronal surface of the enamel and light-cured for 20 seconds. Then, each sectioned sample was stored in distilled water until mounting to exclude the teeth with cracks, fracture lines, caries, developmental anomalies, and existing restorations.

Preparation of Antioxidants (Fig. 1)

All the antioxidants were water-soluble except alpha tocopherol, which was soluble in ethyl alcohol, and lycopene, which was prepared in chloroform.

Division of Groups

All 120 samples were divided into two control groups (n = 20), that is, positive control group (n = 10) and negative control group (n = 10), and five experimental groups (n = 100) such that each experimental group had 20 samples. All the samples except positive control group were bleached with 37.5% hydrogen peroxide bleaching gel (Pola Office, SDI South East Australia) in 3 sessions for each consecutive 8 minutes. The gel was completely rinsed off with distilled water. Except the control groups, experimental groups were further subdivided into subgroups on the basis of antioxidant treatment. This was then followed by building of composite cylinder on the labial surface of each sample in all the groups.

Thus, the positive control group remained unbleached and the negative control group was bleached but received no antioxidant treatment.

Antioxidant Treatment

In the experimental groups, after bleaching with 37.5% hydrogen peroxide, antioxidant treatment was done with 10 mL of antioxidant solution applied on enamel surfaces of all 100 samples, as an irrigating solution, for 10 minutes with a flow rate of 1 mL/min with the 10 mL syringe (Table 1).

Bonding Procedure (Fig. 2)

A piece of plastic hollow cylinder 2 mm in diameter and 4 mm in height was positioned in the center of the flattened portion of enamel of all the samples. Then, 37% phosphoric acid gel (Ivoclar Eco-Etch) was applied on the demarcated portion of enamel of all the samples. Then, the etchant was rinsed with water syringe for 5 seconds and dried with three-way syringe. This was followed by application of bonding agent (Ivoclar Tetric N bond) with the composite resin to the bleached enamel.

Preparation of Antioxidants (Fig. 1)

All the antioxidants were water-soluble except alpha tocopherol, which was soluble in ethyl alcohol, and lycopene, which was prepared in chloroform.

Note: The enamel surface of both the control groups and experimental groups was thoroughly rinsed off with distilled water for 30 seconds and subjected to bonding procedure.

Table 1 Division of samples on the basis of antioxidants used

<table>
<thead>
<tr>
<th>Experimental groups</th>
<th>Antioxidants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 3 (n = 20)</td>
<td>Bleached + 10% sodium ascorbate</td>
</tr>
<tr>
<td>Group 4 (n = 20)</td>
<td>Bleached + 10% alpha tocopherol</td>
</tr>
<tr>
<td>Group 5 (n = 20)</td>
<td>Bleached + 5% proanthocyanidin</td>
</tr>
<tr>
<td>Group 6 (n = 20)</td>
<td>Bleached + 10% green tea extract</td>
</tr>
<tr>
<td>Group 7 (n = 20)</td>
<td>Bleached + 10% lycopene</td>
</tr>
</tbody>
</table>

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applicator tip, gentle drying, and curing for 10 seconds using a Light Curing Unit (Woodpecker led curing light). Then, two increments of 2-mm diameter of composite resin (Ivoclar Te-Econom plus) were placed, each was light cured for 40 seconds separately. Composite resin cylinder of 2 mm in diameter and 4 mm in depth was built. The piece of plastic cylinder was cut and removed. The samples were stored in distilled water till testing for shear bond strength.

Testing Procedure (► Fig. 3)
The samples were tested for the shear bond strength under Universal Testing Machine (Shimadzu, Japan). A knife edge shearing rod with a crosshead speed of 0.5 mm/min was used. The force was applied perpendicular at the interface of enamel surface and the bonded composite cylinder. The load at failure is recorded by laboratory tech notebook software version 6.3. The shear bond strengths of the specimens was calculated and expressed in MPa.

After that statistical analysis was performed using one-way ANOVA and posthoc Tukey’s HSD test.

Results
The present in vitro study was designed for evaluation of the effect of various antioxidants on the shear bond strength of the composite resin to the bleached enamel.

Significant differences were observed among the study groups ($p < 0.05$). Groups I and II demonstrated the highest and lowest mean shear bond strength values, respectively. The samples that were treated with 10% sodium ascorbate (group III) demonstrated significantly higher mean shear bond strength than other experimental groups as shown in ► Table 1 ($p < 0.05$). No significant differences were found between 10% alpha tocopherol (group IV) and 10% proanthocyanidin (group VI) and also comparison between green tea extract (group V) and 5% lycopene (group VII) showed nonsignificant difference ($p > 0.05$), as shown in ► Table 2 (see also ► Fig. 4).

Discussion
The long-term durability of various restorative materials is compromised due to the deleterious side effects of bleaching procedures, possibly credited to the residues from hydrogen peroxide degradation that are known to have low molecular weight. This facilitates their penetration into the enamel tooth surface, affecting the polymerization of adhesive systems and resin composite. $^2$

Thus, to reverse the reduced bond strength, antioxidants were used prior to the composite bonding, as there action takes place by scavenging the residual peroxide radicals, which was also stated by Lai et al, in that use of antioxidants could reverse the deterioration caused by inclusion of peroxide ions.$^4$

In the present study, intragroup comparison of various antioxidants showed that 10% sodium ascorbate (group III) caused maximum reversal of shear bond strength (139.45 ± 10.16) when compared with other experimental groups.

Sodium ascorbate, being a reducing agent, scavenges the free radical through the mechanism of passive detoxification, which is accomplished by donating two high-energy electrons. The results of this study were in accordance with previous investigations done by Lai et al.$^4$ Also, being water-soluble with low-molecular-weight of 198.11 g/mol enables sodium ascorbate to penetrate better.$^5$

Even 10% alpha tocopherol (group IV) showed significantly higher bond strength values as compared with both 10% green tea extract (group V) and 5% lycopene (group VII). This inference was supported by Ratih et al.$^6$

Water insolubility and presence of alcohol in the composition of 10% alpha tocopherol could be credited enough for
depicting the increased shear bond strength values. This inference was supported by Sasaki et al.\(^7\)

However, the difference between group IV (10% alpha tocopherol) and group VI (10% proanthocyanidin) were nonsignificant with value \(p > 0.05\). This inference was not supported by a previous study done by Manoharan et al and Vidhya et al, who concluded that 10% proanthocyanidin showed significantly higher results as compared with 10% alpha tocopherol.\(^8\)

Results of the present study revealed that the reversal of mean shear bond strength done by 10% proanthocyanidin (group VI) was much less than that of 10% sodium ascorbate (group III), which could be due to the higher molecular weight of tannins (500–3,000 g/mol),\(^9\) the category to which proanthocyanidin belongs. This inference was favored by Arumugam et al.\(^2\)

Intragroup comparison of the present study revealed that group VI (10% proanthocyanidin) showed significantly higher shear bond strength value \(p < 0.05\) as compared with the group V (10% green tea) and group VII (5% lycopene).

Late et al in his study inferred that 10% proanthocyanidin is a natural collagen cross-linker that can increase collagen synthesis and decrease the degradation of collagen, all of which strengthen the properties of resin bond. Thus, this inference could be credited to the more effective antioxidant property of proanthocyanidin than 10% lycopene.\(^10\)

It was speculated that higher concentrations of green tea applied for longer period of time may result in a greater

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean (N)</th>
<th>SD</th>
<th>(F)</th>
<th>(p)-Value</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive control (group I)</td>
<td>174.5</td>
<td>24.54</td>
<td>109.677</td>
<td>0.00(^a)</td>
<td>SIG</td>
</tr>
<tr>
<td>Negative control (group II)</td>
<td>42</td>
<td>10.32</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10% sodium ascorbate (group III)</td>
<td>139.45</td>
<td>10.16</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10% alpha tocopherol (group IV)</td>
<td>108.1</td>
<td>13.08</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>10% green tea extract (group V)</td>
<td>78</td>
<td>15.04</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10% proanthocyanidin (group VI)</td>
<td>97.9</td>
<td>17.58</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5% lycopene (group VII)</td>
<td>70.2</td>
<td>14.22</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>100.31</td>
<td>38.31</td>
<td></td>
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</tr>
</tbody>
</table>

**Table 2** Demonstrates the intragroup mean and SD values of shear bond strength (N) for different groups by using one-way ANOVA.

*Abbreviation: SD, standard deviation.*

\(^a\)The mean difference is significant at the 0.05 level.

**Fig. 4** Demonstrate the mean shear bond strength comparison of all the groups.
reversal of bond strength values in bleached enamel. This could be attributed to the presence of catechins in the green tea, such as EGCG and EGC, which are potent antioxidants. This inference was supported by a study done by Ozelin et al.

The bond strength values obtained for 5% lycopene (group VII) were not statistically significant as compared with group V (10% green tea extract), which had mean shear bond strength value of 78 ± 15.04.

The limited diffusion potential across the enamel surface enables lycopene to trap free radicals, which could be due to its least water solubility. This also explains its lowest bond strength value, that is, 70.2 ± 14.22, among the antioxidant group, as concluded by Dhingra et al.

However, a study done by Khamverdi et al was not in favor of our study, who concluded that herbal antioxidants were 20 times better than the sodium ascorbate when they evaluated the effect of herbal antioxidants on the shear bond strength of composite resin to the bleached enamel.

So, the need of the hour is to conduct further studies to evaluate the antioxidant potential of various herbal and synthetic antioxidants for the purpose of determining the stability of their activity by reducing their application time and increasing their concentration in various solution and gel formulations. Moreover, failure mode and scanning electron microscope evaluations of enamel composite interface should be performed in future studies.

**Conclusions**

Within the limitations of the study, it could be concluded that bleaching with 37.5% HP adversely affected the enamel composite bond strength when the bonding procedure was performed immediately after bleaching, although none of the antioxidants were able to completely reverse the mean shear bond strength to the base line value. However, 10% sodium ascorbate caused maximal reversal of the shear bond strength.

**Conflict of Interest**

None declared.

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