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Effect of Grape Seed Extract on the Bond Strength of Bleached Enamel

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Clinical Relevance

Bleached enamel is not conducive to immediate composite restoration. The use of oligomeric proanthocyanidin complexes as antioxidants enables an immediate composite restoration post bleaching.

SUMMARY

Objective: This *in vitro* study assessed the neutralizing effect of grape seed extract (oligomeric proanthocyanidin complexes [OPCs]) on the bond strength of bleached enamel.

Materials and Methods: Labial enamel surfaces of 70 extracted human maxillary central incisors were randomly divided into four groups based on the antioxidant used as follows: Group I (n=20): bleaching with 38% hydrogen peroxide gel for 10 minutes, without the use of an antioxidant; Group II (n=20): bleaching followed by the use of 10% sodium ascorbate solution; Group III (n=20): bleaching followed by the use of 5% proanthocyanidin solution; and Group IV (n=10): control group in which no bleaching was done. Groups I, II, and III were further subdivided into two subgroups of 10 teeth each, based on whether composite build-up was done immediately (subgroup A) or after a delay of 2 weeks (subgroup B) post bleaching. Shear bond strength of the specimens was tested under a universal testing machine. The data were tabulated and statistically analyzed.

Results: Significantly higher shear bond strength values were observed in teeth treated with 10% sodium ascorbate (Group II) and 5% proanthocyanidin (Group III) as compared with the control group (Group IV). Among the antioxidants used, teeth treated with proanthocyanidin showed significantly higher shear bond strength values than those treated with sodium ascorbate.

Conclusion: It can be concluded that the use of grape seed extract prior to bonding procedures on bleached enamel completely neutral-

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izes the deleterious effects of bleaching and increases the bond strength significantly.

INTRODUCTION

Increasing interest in esthetic dentistry has resulted in the widespread practice of vital bleaching.[1] Vital bleaching is a safe and well-accepted procedure for the treatment of both surface and intrinsic staining of teeth.[2] Bleaching agents used contain high concentrations of carbamide peroxide (35% to 37%) or hydrogen peroxide (30% to 38%). Hydrogen peroxide undergoes ionic dissociation to give rise to the formation of free radicals such as nascent oxygen, hydroxyl radical, per-hydroxyl, and superoxide anions when they are applied to dental structure.[3] These free radicals are highly reactive and hence reach out for electron-rich regions of pigment inside the dental structure, breaking down the large pigmented molecules with conjugated double bonds involving carbon, nitrogen, and oxygen atoms into smaller, less pigmented ones.[3,4] However, numerous studies have shown that hydrogen peroxide and carbamide peroxide can adversely affect the bond strength of composite to the acid-etched enamel when bonding is performed immediately after the bleaching process; this is attributed to the presence of residual peroxide that interferes with resin attachment and inhibits resin polymerization.[5-7]

Some techniques have been suggested to solve the clinical problems related to post bleaching compromised bond strength. Barghi and Godwin treated bleached enamel with alcohol before restoration,[7] Cvitko and others proposed removal of the superficial layer of enamel,[8] and Kalili and Sung and others suggested the use of adhesives containing organic solvents.[9,10] However, the general approach is to postpone any bonding procedure for a period after bleaching, because the reduction in bond strength has been shown to be temporary.[11] The waiting period for bonding procedures after bleaching has been reported to vary from 24 hours to 4 weeks.[12-14]

To overcome this delay in bonding, several studies have proposed the use of antioxidant agents like 10% sodium ascorbate after the bleaching procedure. Sodium ascorbate is a neutral, nontoxic, and biocompatible antioxidant that when used as a 10% solution can reverse the reduced bond strength of bleached enamel.[1,2,5,6,15,16] Other naturally occurring antioxidants such as grape seed extract contain oligomeric proanthocyanidin complexes (OPCs) that have free radical scavenging ability, which is shown to be 50 times more potent than sodium ascorbate.[17,18] However, no studies on the effects of proanthocyanidin on bleached enamel can be found in the literature. Hence the aim of this *in vitro* study was to evaluate and compare the effects of 10% sodium ascorbate vs 5% proanthocyanidin on the bond strength of bleached enamel.

METHODS AND MATERIALS

Preparation of Solutions

Two solutions were prepared for this study: 1) 10 g of sodium ascorbate (s d fiNE cHEM Limited, Mumbai, India) was dissolved in 100 mL of distilled water to make 10% sodium ascorbate; and 2) 5 g of grape seed extract in the form of powder (Puritans Pride Inc, Oakdale, NY, USA) was collected from the capsules and dissolved in 100 mL of distilled water to make 5% proanthocyanidin solution.

Specimen Preparation

Seventy recently extracted human maxillary central incisors were collected. Labial enamel surfaces were flattened with 600 grit silicon carbide paper (Moyco Precision Abrasives, Montgomeryville, PA, USA) and their roots were embedded in an acrylic resin block, keeping only the coronal portion exposed. The labial enamel surfaces of 60 specimens were bleached with Opalescence Xtra Boost (38% hydrogen peroxide gel, Ultradent Products, Inc, South Jordan, UT) for 10 minutes according to manufacturers' instructions. The bleaching gel was completely rinsed off with water. Ten teeth served as controls (Group IV) and did not receive any bleaching treatment. The 60 bleached specimens were randomly divided into three groups of 20 teeth each (Group I, II, and III), depending on the type of antioxidant used. They were further subdivided into subgroups A and B based on the storage period before composite build-up. The distribution of specimens and the study groups are listed in Table 1.

Group I (n=20)

- **Group IA (n=10):** Immediately after bleaching and rinsing, the labial surface of each specimen was etched with 37% phosphoric acid (Total Etch etching gel, Ivoclar Vivadent, Schaan, Liechtenstein) for 15 seconds, rinsed with water for 20 seconds, and bonded with Adper Single Bond (3M ESPE, Dental Products, St Paul, MN, USA). This process was followed by composite build-up of 3 mm diameter and 5 mm height (Filtek Z350, 3M ESPE, Dental Products).
- **Group IB (n=10):** After bleaching, the specimens were stored in distilled water for 2 weeks. Then they were etched with 37% phosphoric acid and

Table 1: *Distribution of Specimens and Study Groups*

Group	Antioxidant Used	Subgroup	Composite Build-up
Group I (n=20)	None	A (n=10)	Done immediately
Bleached specimens		B (n=10)	Done 2 weeks after bleaching
Group II (n=20)	10% sodium ascorbate solution	A (n=10)	Done immediately
Bleached specimens		B (n=10)	Done 2 weeks after bleaching
Group III (n=20)	5% proanthocyanidin solution	A (n=10)	Done immediately
Bleached specimens		B (n=10)	Done 2 weeks after bleaching
Group IV (n=10) No bleaching	None	None	Done immediately

bonded with Adper Single Bond; composite build-up followed.

Group II (n=20)

- **Group IIA (n=10):** Immediately after bleaching and rinsing, the labial surfaces of the specimens were treated with 10% sodium ascorbate solution for 10 minutes and rinsed. The surfaces were etched with 37% phosphoric acid and bonded with Adper Single Bond, followed by composite build-up.
- **Group IIB (n=10):** After bleaching, the specimens were stored in distilled water for two weeks**.** Then they were treated with 10% sodium ascorbate solution for 10 minutes. The surfaces were etched with 37% phosphoric acid and bonded with Adper Single Bond; composite build-up followed.

Group III (n=20)

- **Group IIIA (n=10):** Immediately after bleaching and rinsing, the labial surfaces of the specimens were treated with 5% proanthocyanidin solution for 10 minutes and rinsed. The surfaces were etched with 37% phosphoric acid and bonded with Adper Single Bond; this was followed by composite build-up.
- **Group IIIB (n=10):** After bleaching, the specimens were stored in distilled water for 2 weeks. Then they were treated with 5% proanthocyanidin solution for 10 minutes. The surfaces were etched with 37% phosphoric acid and bonded with Adper Single Bond; this was followed by composite build-up.

Table 2: *Comparison of Mean Shear Bond Strength of Different Study Groups*

Group	Mean ± SD, MPa	Overall *p*-Value	Significant Groups
IA	22.66 ± 1.79	<0.001 (sig.)	IIIB vs IA, IB, IIA, IIB, IIIA, IV
IB	32.26 ± 1.45		IIIA vs IA, IB, IIA, IIB, IV
IIA	34.51 ± 1.29		IIB vs IA, IB, IIA, IV
IIB	38.29 ± 1.62		
IIIA	43.44 ± 1.41		IIA vs IA
IIIB	47.96 ± 1.44		IB vs IA
IV	33.33 ± 1.27		IV vs IA

Group IV (n=10)—These specimens were not subjected to bleaching and served as controls. The labial enamel surfaces were etched with 37% phosphoric acid and bonded with Adper Single Bond; composite build-up followed.

All specimens were stored in distilled water for 24 hours before shear bond strength (SBS) testing was performed in a universal testing machine (LR 100K, Lloyd Instruments, Largo, FL, USA). Data were tabulated and statistically analyzed. Kruskal-Wallis one-way analysis of variance (ANOVA) was used to calculate the *p*-value. Mann-Whitney U-test followed by the Bonferroni correction method was used to identify significant groups at the 5% level.

RESULTS

The results of this study are shown in Table 2. Graphic representation of the comparison of mean SBS between groups is given in Figure 1.

When comparing the subgroups A (immediate composite build-up), Group IIIA (5% proanthocyanidin) showed significantly higher SBS values (43.44 ± 1.41) than Group IIA (10% sodium ascorbate; 34.51 ± 1.29), Group IV (unbleached control; 33.33 ± 1.27), and Group IA (bleached, without antioxidant; 22.66 ± 1.79). Group IIA showed significantly higher SBS values than Group IA. No significant

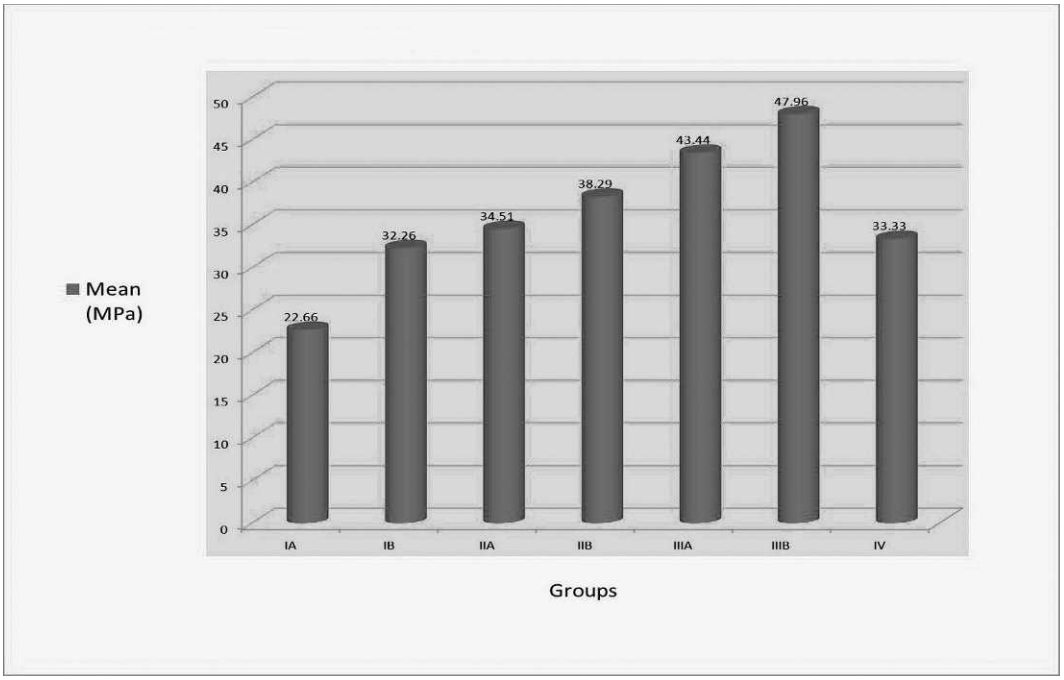

Figure 1. *Graphic Representation of the Comparison of Mean Bond Strength Between Different Study Groups.*

difference in bond strength values was observed between Group IIA and Group IV. Group IV (unbleached control) showed significantly higher SBS values (33.33 ± 1.27) than Group IA (22.66 ± 1.79). Group IA (bleached, without antioxidant) recorded the lowest mean SBS values compared with all other subgroups ($p<0.05$).

When comparing the subgroups B (composite build-up done two weeks after bleaching), Group IIIB (5% proanthocyanidin) showed significantly higher SBS values (47.96 ± 1.44) than Group IIB (10% sodium ascorbate; 38.29 ± 1.62), Group IV (unbleached control; 33.33 ± 1.27), and Group IB (bleached, without antioxidant; 32.26 ± 1.45). Group IIB showed significantly higher SBS values than Group IV and Group IB. No significant difference in bond strength values was noted between Group IB (32.26 ± 1.45) and Group IV (33.33 ± 1.27). Group IB (bleached, without antioxidant) recorded the lowest mean SBS values compared with the other subgroups ($p<0.05$).

Both 10% sodium ascorbate (Group II) and 5% proanthocyanidin (Group III) groups showed significantly higher SBS values than the control group. Among the antioxidants used in this study, 5% proanthocyanidin showed significantly higher shear bond strength values than 10% sodium ascorbate in both immediate and delayed composite build-up subgroups.

DISCUSSION

Vital tooth bleaching procedures are the most commonly used conservative and effective treatment options to treat discolored teeth.[19] *In vitro* bond strength studies using Adper Single Bond (3M ESPE, Dental Products) have reported values in the range of 30 to 35 MPa.[19-21] Lai and others in 2002 showed that bond strengths were reduced by about 25% when bonding was performed to carbamide peroxide bleached enamel (24.0 ± 5.1 MPa) compared with unbleached controls (32.0 ± 6.0 MPa).[20] Several studies have demonstrated that reduction in bond strength of composite post bleaching is due to residual oxygen that is released from the bleaching agent and interferes with resin infiltration into etched enamel and inhibits the polymerization of resin.[9,13,14] Titley and others in 1991 evaluated the scanning electron microscope (SEM) images of interfaces between resin and bleached enamel and observed fragmented and poorly refined resin tags that penetrated to a lesser depth when compared with unbleached controls.[14] Also the entrapment of peroxide ions into the bleached enamel resulted in a resin-bleached enamel interface that was granular and porous with a bubbled appearance.[14,15] Further, changes in organic substance, loss of calcium, and a decrease in micro hardness added to this effect.[22,23]

It has been shown that during bleaching with hydrogen peroxide, hydroxyl radicals in the apatite lattice are substituted by peroxide ions, resulting in the formation of peroxide-apatite. After a two-week storage period, peroxide ions decompose and substituted hydroxyl radicals re-enter the apatite lattice, resulting in elimination of the structural changes caused by the incorporation of peroxide ions.[24] Lai and others (2002) showed that the inclusion process of peroxide ions might be reversed by the use of an antioxidant such as sodium ascorbate.[20]

Ascorbic acid and its salts have a proven safety record for their use as antioxidants in the food industry and are capable of reducing a variety of oxidative compounds, especially free radicals.[1,2,6] It is probable that by restoring the altered redox potential of the oxidized bonding substrate, sodium ascorbate allows free radical polymerization of the adhesive to proceed without premature termination, thus reversing the compromised bonding.[1,2,6,15,16] In this study, the salt of ascorbic acid, sodium ascorbate, was used to prevent the potential double-etching effect of this acid on etched teeth.[16] In the present *in vitro* study, the use of sodium ascorbate (Group II) as an antioxidant has reversed the deleterious effects of 38% hydrogen peroxide; this is shown by an increase in shear bond strength when compared with Group I, in which no antioxidant was used prior to bonding. The results are in concurrence with the findings of previous studies done by Kimyai,[2] Bulut,[5] and Lai and others.[20]

Although many studies have shown the efficacy of sodium ascorbate in the reversal of reduced bond strength to bleached enamel, there is still a paucity of research on OPCs as viable alternatives to sodium ascorbate. Hence in this study, emphasis was placed on the use of OPCs as an antioxidant before the bonding procedure on bleached enamel was performed.

Proanthocyanidins are high molecular weight polymers that comprise the monomeric flavan-3-ol (+) catechin and (-) epicatechin. Proanthocyanidins are found in high concentrations in natural sources such as grape seed extract, pine bark extract, cranberries, lemon tree bark, and hazelnut tree leaves.[17] As a naturally occurring plant metabolite, it has been proven to be safe as an antioxidant in various clinical applications and dietary supplements.[17,25] Therapeutic applications of OPCs in the field of medicine for the treatment of various vascular disorders are well documented. These compounds have also been reported to demonstrate antibacterial, antiviral, anticarcinogenic, anti-inflammatory, and antiallergic properties.[17,18] *In vitro* studies have confirmed that the free radical scavenging ability and the antioxidant potential of OPCs are 50 times greater than those of vitamin C and 20 times greater than those of vitamin E.[17,18] Future experiments comparing different concentrations and application times of OPCs and sodium ascorbate are required to evaluate differences between the two compounds in these aspects.

This study shows that treatment with 5% proanthocyanidin (Group III) increases bond strength significantly compared with Group I, Group II, and Group IV, both when bonding is performed immediately and after storage in distilled water for two weeks following bleaching. This could be attributed to the following:

1. The specificity of OPCs for hydroxyl free radicals
2. The presence of multiple donor sites on OPCs that trap superoxide radicals
3. The esterification of (-) epicatechin by gallic acid in OPCs, which enhances the free radical scavenging ability[17,18]

In the present study, treatment with OPCs gave bond strengths superior to that of unbleached enamel. Hence future research can be carried out on the use of these OPCs as surface treatment agents to improve dentin bond strength.

CONCLUSIONS

Under the limitations of this *in vitro* study, it can be concluded that

1. Treatment of the bleached enamel surface with 5% proanthocyanidin or 10% sodium ascorbate reverses the reduced bond strength and may be an alternative to delayed bonding, especially when restoration is to be completed immediately after bleaching.
2. The use of grape seed extract as an antioxidant yields significantly greater enamel bond strength than that of 10% sodium ascorbate.

Conflict of Interest

The authors have no financial interest in any of the companies or products mentioned in this article.

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