

Original Article

The effect of 10% alpha-tocopherol solution and 5% grape seed extract on the microhardness and shear bond strength to bleached dentin

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ABSTRACT

Background: The aim of the study was to evaluate the effect of 10% alpha-tocopherol and 5% grape seed extract on the microhardness and shear bond strength (SBS) to bleached human dentin.

Materials and Methods: This *in vitro* study was done on 200 extracted premolars which were decoronated and grinded to get flat dentin surface occlusally. They were divided into four groups: (a) bleaching, (b) bleaching and application of alpha-tocopherol, (c) bleaching and application of grape seed extract, and (d) control. Groups were further subdivided into Subgroups I and II ($n = 30$) based on storage period before building with composite and were then tested for microhardness and SBS determination. The data thus obtained was subjected to statistical analysis which was performed using ANOVA test and *post hoc* Tukey's test. The significance for the entire statistical test was predetermined at $P < 0.05$.

Results: The results showed that the microhardness values were minimum in Group A (immediately after bleaching) and maximum in control group. Comparison of data using one-way ANOVA showed that the P value was highly significant ($P < 0.001$) among the groups. The intergroup comparison of SBS using *post hoc* Tukey's tests revealed that the P value was significant ($P < 0.05$) when the comparison was done between the Group A and Group C and Group B with Group D immediately after bleaching.

Conclusion: Adverse effects of bleaching can be reversed with the application of 10% alpha-tocopherol and 5% grape seed extract over the dentinal surface microhardness and SBS.

Key Words: Alpha-tocopherol, grape seed extract, hydrogen peroxide, tooth bleaching agents

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INTRODUCTION

A major esthetic problem in dentistry that often requires treatment is the discoloration of the tooth. The two main causes of discoloration of the tooth are extrinsic and intrinsic.^[1] Intrinsic discoloration in endodontics is mainly due to nonvital tooth. A minimal invasive treatment for such teeth is bleaching which permits a successful esthetic outcome while conserving the tooth structure.^[2]

The bleaching agents mostly used in endodontically treated teeth are hydrogen peroxide (H_2O_2),^[3] carbamide peroxide,^[4] and sodium perborate,^[5] alone or in combinations. Although bleaching agents clearly result in a lighter color of teeth, they have side effects such as changes in tooth surface roughness, increase in tooth porosity, decrease in hardness, increase

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in microleakage, and changes in the mechanical properties of tooth structure, including fracture resistance and fracture toughness.^[3,4,6] Studies have shown that the effect of H₂O₂ on dentine is likely a result of both its strong oxidizing action and its low pH. Using the microhardness tester, studies have shown that exposure to 30% H₂O₂ for 15 min or less was sufficient to cause a decrease in the microhardness of the enamel and dentin.^[7]

The decrease in bond strength of composite resin to enamel and dentin occurs because of the oxygen released, interferes with infiltration of resin tags into the pores of the etched dentin, and also this oxygen acts as an inhibitor that interferes with resin polymerization through the free radical mechanism.^[8] Several studies have proven that this decreased bond strength of resin to bleached dentin can be reversed by the either delaying the bonding procedure for a week or two or by the application of antioxidants such as ascorbic acid, epigallocatechin-3-gallate, sodium ascorbate, alpha-tocopherol, and grape seed extract (proanthocyanidin).^[9-13] Two potent antioxidants that have free radical scavenging abilities are grape seed extract and alpha-tocopherol. Of these two antioxidants, alpha-tocopherol is the most active component of the Vitamin E complex. It is the powerful antioxidant in the lipid phase of the human body.^[12] Grape seed extract contains oligomeric proanthocyanidin complexes that have free radical scavenging ability, which is shown to be fifty times more potent than sodium ascorbate. Proanthocyanidin stabilizes and increases the cross-linkage of Type I collagen of fibrils by hydroxylation of proline which increases the mechanical properties of dentin.^[13]

Hence, a study was needed to be conducted to know the efficiency of these two potent antioxidants in reversing the adverse effects of bleaching to the dentin prior to any restorative treatment. Thus, the purpose of this study was to evaluate the effect of 10% alpha-tocopherol solution and 5% grape seed extract on the microhardness and shear bond strength (SBS) to human dentin submitted to bleaching with 35% H₂O₂.

MATERIALS AND METHODS

Prior to the beginning of this *in vitro* study, ethical committee clearance was obtained from the institutional board of ethical committee. Wilcoxon signed-rank test and Kruskal–Wallis test were utilized

to compare the mean microhardness and SBS values among the various groups and subgroups. The total sample calculated for this study was 200 to estimate the difference in the proportion of poor results between groups with 80% power and 95% confidence.

The sound human premolars without any restorations, fractures, hypoplastic defects, and which were extracted for orthodontic purposes were included in this study. The teeth with cracks or fractures, malformations, carious lesions, restorations, or erosions were excluded from the study. The extracted teeth were thoroughly washed in the running water immediately to remove any blood and adherent tissue. After scaling, the teeth were washed with fluoride-free pumice slurry and rinsed in normal saline and were immersed in 0.1% thymol solution; 24 h before the experiment, the teeth were retrieved from thymol, rinsed, and stored in distilled water.

Preparation of solutions

Two solutions were prepared for this study:

- Five grams of grape seed extract in the form of powder (Good N Natural, Bohemia, NY 11716 USA) was collected from the capsules and dissolved in 100 ml of distilled water to make 5% proanthocyanidin solution
- Ten grams of alpha-tocopherol gel (DI-a-Tocopherol acetate, LOBA CHEMIE, Mumbai, India) was mixed with 100 ml of ethyl alcohol to prepare 10% alpha-tocopherol solution.

The roots of all 200 selected teeth were separated from their crowns at the cemento-enamel junction using a diamond disc. The enamel over the occlusal portion was removed with the help of high-speed carbide bur under water spray. A flat dentin surface was obtained on the occlusal portion of the samples. The surfaces were embedded in the polyvinyl chloride molds of 2.0 cm in diameter, by using self-cure acrylic resin (DPI, India) leaving the dentinal surface uncovered by the resin. The prepared specimens were divided into four groups to receive different procedures, as shown in Figure 1.

Of the 200 samples, 180 samples were bleached with 35% H₂O₂ gel according to the manufacturer's instructions followed by rinsing with distilled water. The samples were exposed thrice for 8 min with the bleaching agent so that the surfaces could be evenly bleached. The 180 bleached dentin surfaces were randomly divided into three groups (Groups A, B, and C) of 60 dentin surfaces each depending on the type of

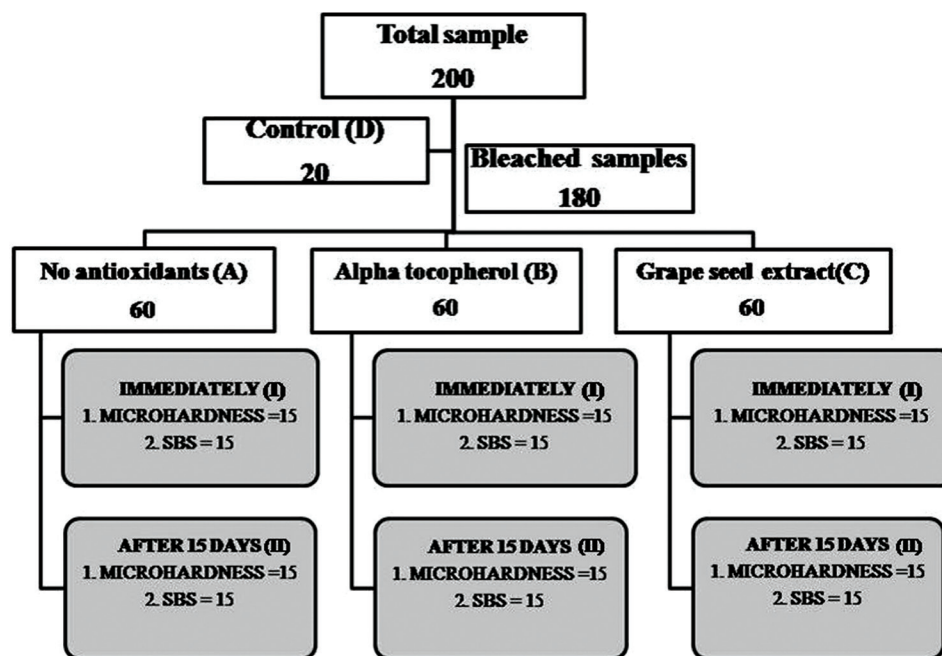


Figure 1: Flowchart showing the sample distribution in four groups.

antioxidant used. These groups were further subdivided into Subgroup I and II ($n = 30$), each based on the time gap (i.e., immediately and after a delay of 15 days) before composite resin buildup. Fifteen samples from each subgroup were randomly selected for the determination of Vickers hardness number (VHN) and the remaining 15 samples were stored in distilled water for 24 h before SBS estimation.

Immediately after bleaching and rinsing, the dentin surface of each specimen in Group B and C was treated with 10% alpha-tocopherol solution and 5% grape seed extract solution, respectively, for 10 min and rinsed with ethanol in Group B and distilled water in Group C.

Composite resin bonding procedure

The remaining 15 samples each from Group A (I and II), Group B (I and II), and Group C (I and II) and 10 samples from control were etched using 37% phosphoric acid (Scotchbond™ Multi-Purpose Etchant, 3M ESPE, St. Paul, MN, USA) for 30 s, washed with water, and dried with filter paper. After etching of the dentin surfaces, bonding agent (Adper™ Single Bond 2 Adhesive, 3M ESPE, St. Paul, MN 55144, USA) was applied and cured as per the manufacturer’s instructions. The customized split metal casing was stabilized using a micropore tape and assembled around the samples to form a circular hole of dimensions 2 mm diameter and 3 mm height followed by placement of the resin

composite (Z100™ Restorative, 3M ESPE, St. Paul, MN, USA) in increments of 1 mm in the hole and photopolymerization was performed for 40 s.

Microhardness test

The samples were subjected to microhardness evaluation using MVK-H1 hardness testing machine (Mitueoyo, USA). Vickers microhardness measurements were made immediately and after 14 days of bleaching and after the application of the antioxidants by three indentations performed. A 300-g load was applied for 15 s.

Shear bond strength test

All the specimens were stored in distilled water for 24 h before SBS testing. The prepared samples were then subjected to SBS evaluation using the Instron Universal Testing Machine (Lloyd Instruments Ltd., UK). A knife-edge shearing rod and a crosshead speed of 1 mm per min were used. The load at failure was recorded by the software in MPa.

The data thus obtained were subjected to statistical analysis using One way ANOVA test, *post hoc* tests for multiple comparisons using Tukey’s test and paired t-test. The significance for the entire statistical test was predetermined at $P < 0.05$.

RESULTS

Table 1 describes the mean microhardness values with their standard deviation and lower and upper

bound values. The mean microhardness in Group AI, AII, BI, BII, CI, CII, and D were 44.4, 43, 62.5, 60, 54.8, 56.4, and 68, respectively. The highest mean microhardness was seen in control group, whereas the lowest was seen in Group A. Table 2 describes an intergroup comparison of the values of microhardness of all groups using *post hoc* Tukey's tests. The results showed that the *P* value among all the groups came out to be significant.

Table 3 depicts the mean SBS to dentin in Groups A to D immediately and after 15 days of the bleaching, in which the highest mean value was seen in Group D and the lowest in Group AI. Table 4 shows the intergroup comparison of SBS

using *post hoc* Tukey's tests which revealed that the *P* value came out to be significant when the comparison was done between the Group A and Group C and Group B and Group D immediately after bleaching. Highly significant results were found when the values of Group A were compared with the values of Group C and D immediately after bleaching, whereas the *P* value was not significant when the SBS values of Group B were compared to Group C and also when the comparison was done between Group C and D. The rest of all the comparisons which were made among the groups 15 days after the bleaching were found to be not significant.

Table 1: Mean microhardness values of Groups A to D at various time intervals in Vickers hardness number

Microhardness	Group	n	Mean	Standard deviation	Standard error	Minimum	Maximum
Immediate (I)	A	15	44.4000	2.74643	0.70912	40.00	48.00
	B	15	62.5333	3.41983	0.88300	58.00	70.00
	C	15	54.8000	3.48876	0.90079	48.00	60.00
	D	10	68.0000	2.86744	0.90676	63.00	72.00
After 15 days (II)	A	15	43.0000	4.24264	1.09545	32.00	48.00
	B	15	60.0667	3.49421	0.90220	53.00	64.00
	C	15	56.4000	3.22490	0.83267	51.00	62.00
	D	10	68.0000	2.86744	0.90676	63.00	72.00

Table 2: Intergroup comparison of microhardness using *post hoc* Tukey's tests

Dependent variable	Group (I)	Group (J)	Mean difference (I-J)	Standard error	P
Immediate (I)	Group A	Group B	-18.13333*	1.15892	<0.001
		Group C	-10.40000*	1.15892	<0.001
		Group D	-23.60000*	1.29571	<0.001
	Group B	Group C	7.73333*	1.15892	<0.001
		Group D	-5.46667*	1.29571	0.001
		Group C	-13.20000*	1.29571	<0.001
After 15 days (II)	Group A	Group B	-17.06667*	1.29608	<0.001
		Group C	-13.40000*	1.29608	<0.001
		Group D	-25.00000*	1.44906	<0.001
	Group B	Group C	3.66667*	1.29608	0.033
		Group D	-7.93333*	1.44906	<0.001
		Group C	-11.60000*	1.44906	<0.001

*The mean difference is significant at the 0.05 level

Table 3: Mean shear bond strength values of groups A to D at various time intervals in MPa

Shear bond strength	Group	n	Mean	Standard deviation	Standard error	Minimum	Maximum
Immediate (I)	A	15	28.73	12.487	3.224	10	48
	B	15	41.60	11.494	2.968	17	60
	C	15	49.47	9.078	2.344	30	62
	D	10	57.60	15.529	4.911	20	73
After 15 days (II)	A	15	49.00	7.919	2.045	32	60
	B	15	51.93	4.862	1.255	43	60
	C	15	56.67	6.619	1.709	45	68
	D	10	57.60	15.529	4.911	20	73

Table 4: Intergroup comparison of shear bond strength using *post hoc* Tukey's tests

Shear bond strength	Group (I)	Group (J)	Mean difference (I-J)	Standard error	P
Immediate (I)	Group A	Group B	-12.867*	4.386	0.025
		Group C	-20.733*	4.386	<0.001
		Group D	-28.867*	4.903	<0.001
	Group B	Group C	-7.867	4.386	0.288
		Group D	-16.000*	4.903	0.010
		Group C	-8.133	4.903	0.356
After 15 days (II)	Group A	Group B	-2.933	3.231	0.801
		Group C	-7.667	3.231	0.095
		Group D	-8.600	3.612	0.094
	Group B	Group C	-4.733	3.231	0.466
		Group D	-5.667	3.612	0.405
		Group C	-0.933	3.612	0.994

*The mean difference is significant at the 0.05 level

DISCUSSION

Dentists have been perplexed by the problem of tooth discoloration because the patient's esthetic expectations have expanded notably in the past few decades. The appearance of dentition is of concern to many people seeking dental treatments. Treatment modalities include microabrasion, macroabrasion, veneering, and placement of porcelain crowns, but they all require cutting of tooth structure.^[14] There are increasing numbers of patients who do not want their teeth to be "cut down" for crowns and prefer an alternative, conservative approach of tooth bleaching.

H₂O₂ is a colorless liquid with a bitter taste and is highly soluble in water to give an acidic solution. H₂O₂ was used as a bleaching agent with concentration of 35%. Its bleaching efficacy is related to the interaction of the bleaching material to the stain molecules and its ability to diffuse into the tooth structure.^[15] Bleaching has also been associated with changes in the biomechanical properties dentine such as microhardness and also causes a substantial reduction in the adhesiveness of composite resin to the dentin.^[3,4,6]

Previous studies showed that the bleaching agents can promote chemical alterations in the composition of the tooth, reducing the quantity of calcium and phosphate in enamel and dentin.^[6,16,17] Some authors in their studies have observed a reduction in dentinal surface microhardness following a bleaching treatment with H₂O₂.^[18-20] In our study also, a similar finding was observed when the microhardness of the samples was tested. It was seen that the highest microhardness was observed in the samples which were unbleached (68 VHN) and it was reduced to a significant value in the bleached samples (44.4 VHN).

The bleaching agent not only affects the surface microhardness of the dentin but also reduces the bond strength of the composite resins to the dentin. The oxygen released during the bleaching procedure interferes with infiltration of resin tags into the pores of the etched dentin and also acts as an inhibitor in resin polymerization through the free radical mechanism.^[21] In this study also, the SBS of the dentin immediately after the bleaching was found to be significantly reduced as compared to the unbleached dentin.

Various methods and the materials have been applied to overcome this reduced hardness and the bond strength of bleached dentin either by delaying the restorative for 2–4 weeks or by applying fluorides, buffering agents, and antioxidants, etc.^[22] One of the methods which involve the delaying of the composite restoration for 7–14 days was found to be successful in various studies. Shinohara *et al.*^[1] concluded in their study that the decrease in SBS values is time dependent. According to them, a delay of 2 weeks in bonding procedures for composite resin restoration is recommended.

The results of the present study showed that the microhardness values of the bleached dentin after 15 days of the bleaching procedure did not show any difference in the VHN values when compared with the values of the immediate group. On the other hand, the SBS values of the bleached dentin after 15 days (Group AII) showed a significant increase, i.e., immediately after bleaching the SBS was 28.73 N, whereas after 15 days, it came out to be 49 N. This seems to be due to the leaching of H₂O₂ from the dentinal tubules during the storage. Few authors have also concluded the similar finding that a 2–4 week

delay is necessary for the increase in the reduced sealing ability of resin composite to the bleached dentin.^[23-26]

The other methods of improving the hardness and SBS of the bleached dentin included the application of antioxidants.^[27] Two such antioxidants which are used in the study are 10% alpha-tocopherol and 5% grape seed extract. In this study, the microhardness of bleached dentin after the application of 10% alpha-tocopherol showed a significant rise in the values immediately after the bleaching (Group B I), i.e., it was 62.3 VHN which was almost equivalent to the microhardness of the unbleached dentin (68 VHN). Two weeks after the application of 10% alpha-tocopherol, the VHN values did not show any significant changes. Hence, the microhardness of the bleached dentin with alpha-tocopherol remained the same as that of its immediate application.

The SBS values did not show any significant increase after the application of alpha-tocopherol on the bleached dentin immediately, whereas after 15 days, the values increased up to 51.93 N which was almost close to the VHN values of the control group. Similar results were found by a study done by Harrison *et al.*,^[26] whereas the study conducted by Whang and Shin^[28] showed different results where they concluded that surface treatment with alpha-tocopherol for 60 s resulted in almost the same bond strength as that of the control and 4-week group without thermal stress. Studies conducted on bleached enamel surface with 5% grape seed extract showed that the antioxidant has the capacity to reverse the reduced bond strength and it could be an alternative to delayed bonding, especially when the restoration is to be completed immediately after bleaching.^[29,30]

The values of bleached dentin after the application of 5% grape seed extract showed an excellent reversal of the SBS immediately and after 15 days of the treatment. The SBS value immediately after bleaching was 49.47 N, and after 15 days, it was 56.67 N which was almost equal to the SBS of unbleached group. The highest SBS was seen after the application of 5% grape seed extract as compared to 10% alpha-tocopherol. Hence, grape seed extract was found to be more effective in reversing the SBS of the bleached dentin. Since this study was an *in vitro* study which have some limitations, as the majority of variables in the oral cavity during bleaching *in vivo* do not hold *in vitro*. In addition, factors such as dentin age, dentin thickness, dentin depth, sclerosis of the

dentinal tubules, and the oral cavity conditions such as the presence of natural saliva, which is an important clinical variable, do not hold *in vitro*; these variables greatly influence the bonding and bleaching procedure of dentin. Hence, further studies comparing the effect of these two antioxidants in *in vivo* are required to evaluate differences between the two compounds.

Based on the observations of this study, both 5% grape seed extract and 10% alpha-tocopherol solutions were found to be effective antioxidants in reversing the adverse effects of the bleaching procedure on the dentin pertaining to the SBS and microhardness and will help in achieving effective bond strength of adhesive restorations. In comparison, 5% grape seed extract solution was proved to be more effective than 10% alpha-tocopherol solution in reversing the SBS of the bleached dentin.

CONCLUSION

Within the limitations, the following conclusions were drawn from the present study – the findings of the present study support that the bleaching procedure with 35% H₂O₂ decreases the dentin surface microhardness and the SBS. To overcome this adverse effect, a delay of 2 weeks is recommended before the bonding procedures for composite resin restoration are done. Application of 10% alpha-tocopherol solution and 5% grape seed extract was found to be effective in reversing the adverse effects of bleaching over the dentinal surface microhardness and SBS. On comparing the both, 10% alpha-tocopherol solution proved more effective in reversing the dentinal surface microhardness, whereas 5% grape seed extract solution was effective in reversing the adverse effects of bleaching procedure on bond strength of dentin/restorative material interface.

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Conflicts of interest

The authors of this manuscript declare that they have no conflicts of interest, real or perceived, financial or nonfinancial in this article.

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