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AN IN VITRO COMPARATIVE STUDY EVALUATING THE EFFECT OF TWO NON-ENZYMATIC ANTIOXIDANTS ON SHEAR BOND STRENGTH OF RESIN BASED COMPOSITE RESTORED TO OFFICE BLEACHED ENAMEL

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ABSTRACT

Aim: To evaluate and compare the effect of 5% grape seed extract solution and 5% green tea extract solution on shear bond strength of resin based composite restored on office bleached enamel. **Materials and methods:** Labial enamel surfaces of 60 freshly extracted human maxillary central incisors were flattened using 600 grit silicon carbide paper. Then specimens were randomly divided into four groups of fifteen samples in each group. (n=15). Group 1: no bleaching (control); Group 2: Bleaching with 35% hydrogen peroxide gel; Group 3: Bleaching, followed by application of 5% grape seed extract (proanthocyanidin); Group 4: Bleaching, followed by application of 5% green tea extract (epigallocatechin gallate). Labial surfaces of all specimens were etched with 37% phosphoric acid and rinsed with water. Then bonding agent was applied and composite buildup was done. Then all samples were subjected to shear bond testing in universal testing machine. **Statistical analysis:** One-way ANOVA was used for multiple group comparison and post hoc Tukey's test for individual group wise comparison. **Results:** Highest shear bond strength values were observed in control group (25.55 $\pm$ 3.49). Among the antioxidants, Group 4 (19.72 $\pm$ 2.28) showed significantly higher shear bond strength values than Group 3 (18.77 $\pm$ 2.05). although there was no statistically significant difference between Group 3 and 4(P = 0.741). Lowest shear bond strength was found in Group 2 (12.14 $\pm$ 2.23).

KEYWORDS: Vital tooth bleaching, Shear bond strength, Antioxidants, Grape seed extract, Green tea extract.

INTRODUCTION

In current era of dentistry tooth discoloration is a factor of utmost concern as more emphasis is being placed on esthetics. In many such cases, treatments of choice are micro abrasion, bleaching, composite resin restoration and porcelain veneer.[1] Increase in the demand for minimally invasive dentistry resulted in widespread practice of vital tooth bleaching, as it is considered as a safe, popular, conservative and well accepted treatment option for discolored teeth.[2] This procedure should be combined with tooth colored restorative procedures in most cases to achieve optimal esthetic results.[3]

It is seen that vital bleaching adversely but transitionally reduces enamel and dentin bond strength when immediate bonding is performed after bleaching. This decreased bond strength is due to the presence of oxygen ions which interfere with polymerization of the resin bonding agent and formation of sufficient resin tags in the etched enamel.[1] Some of the techniques proposed to overcome this clinical problem, are as follows:

- Delay the bonding procedure after bleaching
- Conditioning the bleached enamel with alcohol before restoration[4]
- Removing the surface layer of enamel[5]
- Employing adhesives containing organic solvents[6]
- Surface treatment with Nd:YAG and Er:YAG lasers[7]

Nonetheless, at present, the universal approach to regain bond strength after bleaching procedure is to delay the bonding procedure after bleaching for 4 days to 4 weeks.[8] Another technique to avoid such a delay and enable immediate bonding after bleaching procedures is to apply antioxidative agents. There are some non-enzymatic antioxidants getting popularity in modern dentistry, like Proanthocyanidin (PA), Alpha tocopherol, β- Carotene Ascorbate, Lycopene and Epigallocatechin gallate (EGCG) etc.

Therefore, the present study was carried out to evaluate and compare the effect of 5% grape seed extract solution and 5% green tea extract solution on the shear bond

strength of composite resin restored on office bleached enamel.

METHODOLOGY

Sixty human maxillary central incisor teeth, extracted for periodontal reason were collected. Any tissue remnants and calculus were removed using ultrasonic scaler. All teeth were washed under running water and stored in saline until mounting. Then all teeth were embedded in self-cure acrylic resin blocks (15mm x 15mm x 25mm) till cementoenamel junction (Figure 1). Labial surfaces of all the specimens were flattened using 600 grit silicon carbide paper. Then specimens were randomly divided into **four groups. (n=15)**

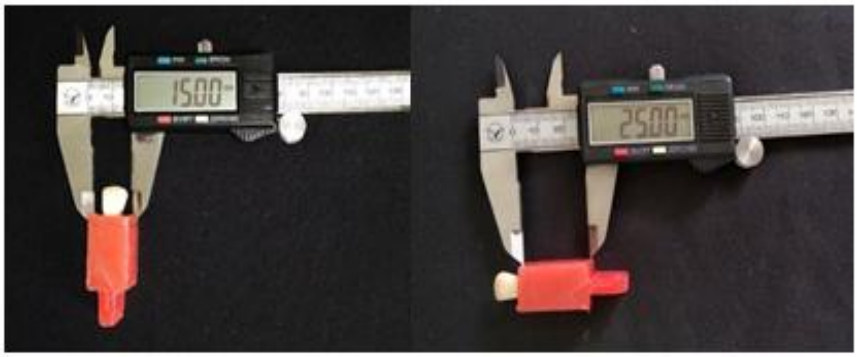

Figure 1: model of 1.5cm X 1.5cm X 2.5cm.

Table 1: Distribution of specimens and study groups.

Groups	Bleaching agent	Antioxidant used	Composite build up with Filtek Z250 XT
Group 1: control (n=15)	None	None	Done immediately
Group 2: no antioxidant used (n=15)	35% hydrogen peroxide gel for 30 mins	None	Done immediately
Group 3: grape seed extract (n=15)	35% hydrogen peroxide gel for 30 mins	5% PA solution for 10 mins	Done immediately
Group 4: green tea extract (n=15)	35% hydrogen peroxide gel for 30 mins	5% EGCG solution for 10 mins	Done immediately

Preparation of antioxidant solution

5grams of grape seed extract in the form of powder were collected and dissolved in 100 ml of distilled water to obtain 5% proanthocyanidin **(PA)** solution. Similarly 5% epigallocatechin gallate **(EGCG)** solution was prepared from green tea extract (Figure 2).

Figure 2: Green tea and grape seed extract.

Bleaching procedure

Labial enamel surfaces of specimens in group 2, 3 & 4 were bleached using Pola office one patient kit (SDI, Victoria, Australia) containing 35% hydrogen peroxide with three applications of ten minutes each. Bleaching gel was completely rinsed off with water and air dried (Figure 3).

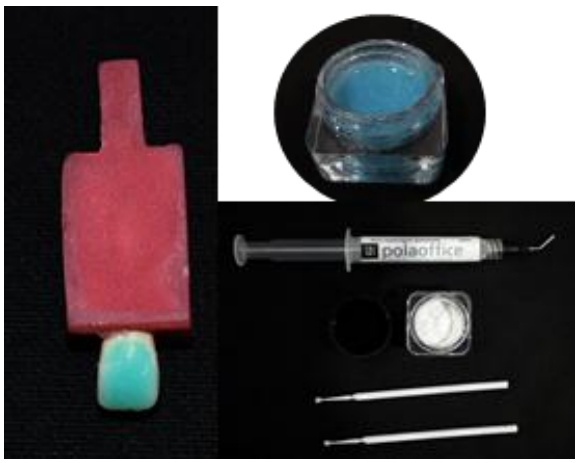

Figure 3: Application of bleaching gel for 30 minutes.

Application of antioxidant

After bleaching procedure, 5% PA and 5% EGCG solution was applied on the prepared enamel surface of each specimen in group 3 and group 4 (Figure 4). Antioxidant solution was added repeatedly to make sure that solution was not getting dried. After 10 minutes specimens were rinsed and air dried.

Figure 4: Application of green tea extract (10 minutes) on samples of Group 4.

Bonding procedure

Enamel surfaces of all specimen in group 1, 2, 3 and 4 were etched with 37% phosphoric acid (Scotchbond multipurpose etchant gel, 3M ESPE, Dental Products, St Paul, MN, USA) for 30 secs, followed by rinsing with water and air dried. Bonding agent (Adper Single Bond 2, 3M ESPE, Dental Products, St Paul, MN, USA) was applied and cured for 30 seconds.

A cylindrical poly vinyl chloride (PVC) mold of 3mm internal diameter and 5mm height was placed vertically at the center of prepared enamel surface. Nanohybrid composite (Filtek Z250 XT Universal Restorative, 3M ESPE, Dental Products, St Paul, MN, USA) of 2mm increment was condensed inside the mold with a teflon coated instrument and cured for 40 seconds (Figure 5). Followed by final increment of 3mm was placed over the first increment to form the total 5mm height and cured for 40 seconds.

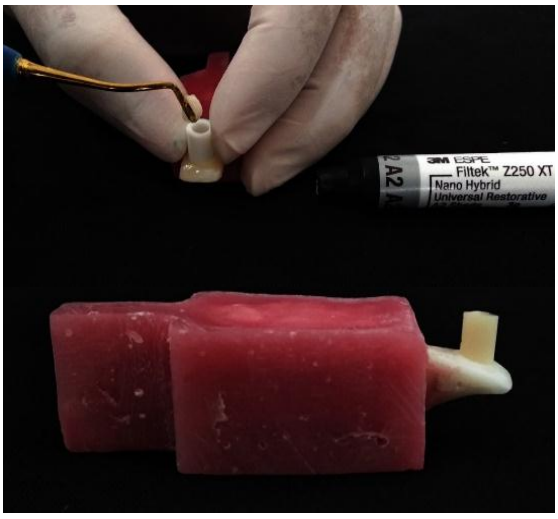

Figure 5: Incremental composite build up using PVC mold.

Then the PVC moulds were cut vertically using no.11 BP blade and removed leaving the 5mm of cylindrical composite build up on labial surface. To make sure complete polymerization, all surfaces of composite resin were cured again for 20 seconds each.

Then all samples were subjected to shear bond testing. Force was applied by a metal knife edge parallel to the long axis of the tooth at the junction between the composite and enamel interface (Figure 6). The shear bond strength was measured in shear mode at a crosshead speed of 0.5mm/min until fracture occurs.

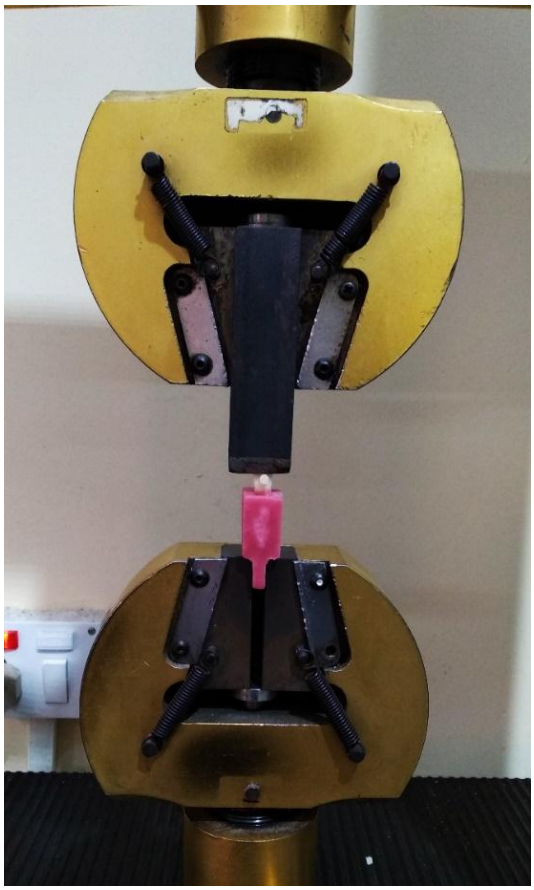

Figure 6: Shear bond testing in universal testing machine.

Statistical analysis

The data was subjected to statistical analysis using one-way ANOVA (Analysis of variance) in which mean was found and with Tukey's post-hoc test the difference in values between the groups were calculated.

RESULTS

Highest shear bond strength values were observed in control group (25.55 $\pm$ 3.49). Lowest shear bond strength was found in Group 2 (12.14 $\pm$ 2.23). Among the antioxidants, Group 4 (19.72 $\pm$ 2.28) showed significantly higher shear bond strength values than Group 3 (18.77 $\pm$ 2.05). Although there was no statistically significant difference between Group 3 and 4(P = 0.741).

Table 2: Mean Shear bond strength between the four groups were compared using one-way ANOVA.

One Way ANOVA								
Shear Bond Strength in Mpa								
Groups	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Min	Max
					Lower Bound	Upper Bound		
Composite (Control)	15	25.55	3.49	0.90	23.62	27.48	20.63	32.06
Bleaching	15	12.14	2.23	0.57	10.90	13.37	8.40	17.12
Bleaching + PA	15	18.77	2.05	0.53	17.63	19.90	14.25	21.16
Bleaching + EGCG	15	19.72	2.28	0.59	18.46	20.99	12.68	22.68

Table 3: Multiple comparison between groups.

Multiple Comparisons						
Dependent Variable: Shear Bond Strength **Tukey HSD**						
(I) GROUP	(J) GROUP	Mean Difference (I-J)	Std. Error	p	95% Confidence Interval	
					Lower Bound	Upper Bound
Composite (Control)	Bleaching	13.41	0.94	**<0.001**	10.91	15.91
	Bleaching + PA	6.78	0.94	**<0.001**	4.28	9.27
	Bleaching + EGCG	5.82	0.94	**<0.001**	3.33	8.32
Bleaching	Bleaching + PA	-6.63	0.94	**<0.001**	-9.12	-4.13
	Bleaching + EGCG	-7.58	0.94	**<0.001**	-10.08	-5.09
Bleaching + PA	Bleaching + EGCG	-0.95	0.94	0.741	-3.45	1.53

DISCUSSION

This in vitro study evaluated the effect of two antioxidants on reversal of deleterious effect of in office vital bleaching, when immediate bonding is planned. Some studies showed that the decrease in bond strength is due to presence of residual peroxide on tooth surface, which interferes with resin bonding and prevents its complete polymerization (Dishman et al., 1994). Others mentioned that vital bleaching will alter the protein and mineral content of the superficial layers of enamel, which may be responsible for reduced bond strength.[9] Titley et al. reported that, in the SEM evaluation of bleached specimens, large areas of enamel surface were resin free and tags were poorly defined and fragmented and penetrated to a lesser depth when compared with those in the unbleached control groups.[10]

To improve the bond strength of bleached enamel to composite, different methods have been proposed in the literature. Some authors have suggested that, the bonding procedure should be delayed by a period varying from 24 hours to three weeks.[11] Barghi and Godwin, treated bleached enamel with alcohol before restoration, Cvitko and others proposed removal of the superficial layer of enamel and Kalili and Sung and others suggested the use of adhesives containing organic solvents and the utilization of antioxidants.[12] Some investigators demonstrated that catalase or catalase like substances can be used as effective adjuncts after bleaching treatment to decrease the residual HP on the bleached teeth (Kum et al., 2004).[12] Among all the methods, the antioxidant treatment has shown immediate improvement in shear bond strength (SBS).[13,14] Naturally occurring antioxidants such as grape seed extract, green tea extract contains oligomeric proanthocyanidin complexes (OPCs) that have free radical scavenging ability.[11] Utilization of natural antioxidants like plant extracts as a viable alternative to chemical and synthetic antioxidants have been reported in recent years. Since, there is a limited information available on the use of the newer natural antioxidant agents like OPCs and epigallocatechin gallate, in this study emphasis was placed on the use of grape seed and green tea extract as antioxidants immediately following the bleaching procedure to reverse the compromised bond strength of composite resin to bleached enamel.

Both the antioxidants were capable of reversing the reduced SBS following bleaching. The reduced SBS in Group 2 when compared to Group 1, 3 and 4 may be due to the residual oxygen layer left behind following the bleaching process which could have interfered with the resin infiltration into etched enamel and inhibited the polymerization of composite resin.

Group 3 and 4 specimens showed significantly higher SBS than that of Group 2 but lesser than that of Group 1. These findings were in accordance with other studies which states that this could be attributed to the specificity of OPCs for hydroxyl free radicals, the presence of multiple donor sites on OPCs that trap superoxide radicals and the esterification of (-) epicatechin by gallic acid in OPCs, which enhances the free radical scavenging activity.

Group 4 specimens showed a slightly higher mean SBS value than that of Group 3 specimens. Although there was no statistically significant difference between these two groups. The difference in the antioxidant activity of grape seed and green tea extract could likely be attributed to their different phenolic compositions.[2] The cardinal antioxidative ingredient in the green tea extract is green tea catechins (GTC), which comprise four major epicatechin derivatives; namely, epicatechin (EC 6.4%), epigallocatechin (EGC -19%), epicatechin gallate (ECG-13.6%) and epigallocatechin gallate (EGCG – 59%).[15] It has been shown that polyphenols in green tea inhibit the oxidative activity in rats up to 72%.[16]

In addition, other recent studies found that the method of application and the chemical composition of the adhesives could affect the efficacy of antioxidant as a reducing agent (Khoroushi and Aghelinejad, 2011; Khoroushi and Saneie, 2012).[17,18] A study by Zahra et al found that the duration of EGCG application did not result in significant differences in shear bond strength values, indicating that this material can provide sufficient antioxidative effect in at least 10 minutes. Selection of 10 minutes application time in the present study was based on this study.[19]

In the present study, the solution form of antioxidant was used. Awdah AS found no significant difference among the different forms of antioxidant (solution and gel). The differences are in the flow ability and the time of application, where the gel form is more convenient in application but the solution form requires less time to work. In clinical conditions, applying the solution form is difficult because of its high flow ability and it requires continuous application, especially if application to more than one tooth is required.[20]

The most frequently ignored guideline in the test protocol is to follow the ISO/TS 11405 specification (2003), that is, "a limitation of the bonding area is important".[21] Hence, to fulfill the criteria PVC molds of 3‑mm internal diameter and 5‑mm height were used.

CONCLUSION

Within the limitations and based on the results obtained, the following conclusions can be drawn from the present in vitro study.

1. Application of 35% hydrogen peroxide as a bleaching agent, significantly decreases bond strength of composite to enamel, if bonding is done immediately.
2. Treatment of the bleached enamel surface with 5% proanthocyanidin or 5% epigallocatechin gallate reverses the reduced bond strength and may be an alternative to delaying bonding procedure after vital bleaching.
3. The use of 5% green tea extract as an antioxidant yields greater bond strength to bleached enamel, than 5% grape seed extract.

Although these antioxidants are not marketed specifically for dental use, but they will play promising role in future restorative and esthetic dentistry.

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