ORIGINAL RESEARCH

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Impact of Different Antioxidants on the Bond Strength of Resin-based Composite on Bleached Enamel—An *In Vitro* Study

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ABSTRACT

Aim: The aim of this *in vitro* investigation was to assess the impact of various anti-oxidants namely 5% proanthocyanidin, 10% sodium ascorbate, 10% alpha tocopherol, and 10% green tea on the bond strength of resin-based composite on bleached enamel.

Materials and methods: One hundred twenty human maxillary central incisors which were freshly extracted for periodontal reasons, having intact labial surface were us ed in this study. Specimens were randomly divided into six groups (n = 20). Group A: negative control–no bleaching treatment, group B–positive control –bleaching, group C–bleaching + 5% proanthocyanidins, group D–bleaching +10% green tea, group E - bleaching +10% alpha –tocopherol, group F–bleaching + 10% sodium ascorbate. Surfaces were etched followed by application of total-etch bonding system, and composite resin cylinders were bonded. Specimens were tested for shear bond strength using the universal testing machine .

Statistical analysis used: The data obtained were subjected to ANOVA and post hoc Tukey's test for statistical analysis.

Results: After using bleaching agents and antioxidants for the different groups, Group A has the highest bond strength and group B has the lowest bond strength. Amongst the antioxidants group D showed significantly higher bond strength as compared group C, group E, and group F.

Clinical significance: Use of antioxidants instantly following the bleaching procedure and before resin bonding reverses the compromised bond strength of composite resin on bleached enamel without sitting tight for a time of one day to one month.

Conclusion: Bleaching of enamel reduced the shear bond strength. All the antioxidants used in this study increased the bond strength of bleached enamel. Among the antioxidant groups, green tea extract showed significantly higher bond strength compared to proanthocyanidin, tocopherol, and sodium ascorbate.

Keywords: Antioxidant, Bleaching, Shear bond strength.

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INTRODUCTION

Tooth staining is generally unprepossessing and mentally traumatizing.[1] Treatment choices for stained teeth incorporate evacuation of surface stains, bleaching, microabrasion, macro abrasion, veneering (direct or indirect) and application of porcelain crowns.[2,3]

Tooth bleaching has been a choice since the late 1870s as it allows a fruitful stylish result at negligible cost while saving the tooth structure. The process of bleaching depends on oxidation-reduction, which discharges oxygen free radicals.[4] Bleaching agents like hydrogen peroxide (H_2O_2), carbamide peroxide, sodium perborate, carbopol, in multitudinous differing focuses have been utilized to evoke esthetically pleasing outcomes.[5,6]

Inconveniences of bleaching may change from postoperative affectability to pulpal disturbance to tooth structure alterations or micro-leakage of current

restorations.[2] Another essential difficulty following bleaching is diminished bond quality of composite pitch to enamel.[7] This diminished bond quality has been attributed to the nearness of oxygen particles that hinder resin polymerization. The decreased shear bond strength may likewise be owing to change in the microstructure of bleached enamel surfaces subsequent to acid etching including lessening of calcium substance of enamel and diminished microhardness, and loss of enamel prisms.[1]

Thinking about this, different techniques were proposed to dodge clinical issues identified with compromised bond strength after bleaching, for example, removal of the shallow layer of enamel, [8] pretreatment of bleached enamel with liquor, use of cement containing natural solvents[9] and use of antioxidants.[10]

Among all the strategies mentioned above, the antioxidant treatment has demonstrated a quick change in the effectiveness of bond strength, though alternative techniques indicated clashing outcomes in recapturing the e ffectiveness of bond strength.[11] Antioxidants kill free radicals by giving one of their electrons, finishing the electron taking the response, and adjusting the redox capability of the bleached surface. Decreased bond strength can be turned around by the utilization of antioxidants, for example, sodium ascorbate, alpha-tocopherol, proan thocyanidin and green tea extract (catechins and epigallatochingallate).[12]

Sodium Ascorbate is a sodium salt of the ascorbic acid, and it is an intense antioxidant for extinguishing receptive free radicals in organic systems.[13] Proanthocyanidin is a grape seed extract, which is a characteristic antioxidant that has the more noteworthy capability of searching oxygen free radicals. Green tea is produced using the camellia sinensis plant. It contains flavanols or catechins which have appeared to have intense antioxidant movement that is a few times higher than that of vitamins C and *E. Alpha–tocopherol* is a ground-breaking antioxidant in the lipid phase of the human body and has been recommended for enhancing composite bonding followed by bleaching.[6] However, comparative assessment of these antioxidants is yet to be evaluated.

So this *in vitro* examination has evaluated the part of antioxidants to be specific: 5% proanthocyanidin, 10% sodium ascorbate, 10% alpha tocopherol and 10% green tea on inversion of bond quality in bleached teeth. The assessment of the bond quality was done by shear bond test utilizing instron widespread testing machine.

MATERIALS AND METHODS

This *in vitro* study was carried out in the Department of Conservative Dentistry and Endodontics, Maharishi Markandeshwar College of Dental Sciences and Research, Mullana (Ambala) Haryana and the evaluation of the samples was carried out using the universal testing machine at Spectro Analytical Lab Ltd, Okhla, New Delhi.

Inclusion Criteria

Human maxillary central incisors, freshly extracted for periodontal reasons with intact labial enamel surfaces, not subjected to pre-treatment with chemicals.

Exclusion Criteria

Dental caries, any previous restorative or endodontic treatment, fractured teeth, attrition, abrasion, erosion and developmental defects or hypoplastic surface.

Sample Preparation

Specimens were randomly divided into six groups (n = 20). Group A–negative control–no treatment, group B: positive control – bleaching, group C–bleaching +5% proanthocyanidins, group D–bleaching +10% green tea, group E–bleaching +10% alpha–tocopherol, group F–bleaching + 10% sodium ascorbate.

A total of 120 specimens were utilized. The teeth were stored in a disinfectant solution of 0.1% thymol prior to the study. Keeping solely the labial enamel surfaces exposed samples were embedded in an acrylic resin block. To attain a flat, homogenous enamel surface the labial surfaces of the samples were polished consecutive with wet 400- and 600-grit carbide papers for thirty sec, while not exposing the dentin in any of the samples and cooled below running water. To eliminate any leftover residues, left by the carbide on the enamel surface, for ten minutes of teeth were washed ultrasonically in distilled and deionized water after polishing.

Distribution of Specimen and Study (Table 1)

Bleaching Treatment

Predetermined volume 0.02 ml of bleaching gel was applied on the enamel surface with the help of bleaching tray. According to the manufacturer's instructions the bleaching procedure was performed for 8 hours daily for 7 days at 37 ± 1°C. The samples were maintained at 37 ± 1°C in the 16 remaining hour's interim in distilled water. Under running water, the bleaching gel was removed from the samples following the bleaching treatment.

Application of Antioxidants

After bleaching, 5% proanthocyanidin, 10% green tea, 10% alpha-tocopherol and 10% sodium ascorbate solution were applied to the surface of specimens in groups C, D, E and

Table 1: Distribution of specimen and study groups.

Group (n = 20)	*Bleaching agent*	*Antioxidant used*	*Preparation of antioxidant*	*Source*
Group A (negative control)	No bleaching treatment	No antioxidant	–	–
Group B (Positive control)	15% Carbamide peroxide	No antioxidant	–	–
Group C	15% Carbamide peroxide	5% proanthocyanidin	5 g of grape seed extract (powder) + 100 ml of distilled water	Grape seed extract, Pukhraj Herbals Mandsaur; India
Group D	15% Carbamide peroxide	10% green tea	10 grams of Green Tea + 100 ml of distilled water	10% green tea (Lipton, Uniliver Pvt Ltd; India
Group E	15% Carbamide peroxide	10% Alpha – Tocopherol	10 grams of Alpha-Tocopherol (powder) + 100 ml of ethanol	Alpha tocopherol, S.D Fine Pvt. Ltd , Bombay; India
Group F	15% Carbamide peroxide	10% Sodium Ascorbate.	10 grams of sodium ascorbate (powder) + 100 ml of distilled water	CDH Sodium L Ascorbate, Ardra chemicals Pvt. Ltd; India

F respectively for 10 minutes and during this exposure the enamel samples were continuously agitated using a micro brush. To compensate for the evaporated solution, fresh solution was applied to the surface every minute. Next, the surfaces were rinsed under running water and gently air-dried.[14]

Bonding Procedures

Thirty-seven percent phosphoric acid for 30 seconds (Pro etch, S.S white) was used to condition the surface of enamel and for 10 seconds washed with spray air/water. Afterward, an absorbent paper was used to dry the surface. According to the manufacturer's instructions, two layers of the adhesive system (Prime and Bond NT) were applied to the conditioned enamel surface, following a light air jet. Polymerization was done using curing light for 10 seconds. The embedded specimens were mounted in an apparatus containing a teflon mold with a circular hole (3 mm in diameter and 5 mm high). Two increments of a composite resin (ESTHET.X HD) were inserted into the mold and each increment was light cured for 40 seconds. After curing, mold was removed and the composite cylinder was additionally light cured for 40 seconds from both the sides, to ensure the maximum polymerization of the resin and enabling a homogeneous stress distribution to the bonding interface during the fracture mode analysis test. Immediately after the bonding procedures, the teeth were stored in distilled water at 37 ± 1°C for 24 hour.

Shear Bond Strength Measurement

The specimens were placed in between the jigs of the universal testing machine, and a pointed shearing rod was placed on the composite resin/tooth interface such that the chiseled model (fixture) of the device would lie perpendicular to the composite cylinders. Force was then applied over the composite cylinders at a crosshead speed of 0.5 mm/minute until the cylinders got detached from the enamel surface. The specimens were mounted and stressed in shear at a rate of 0.5 mm/min using a universal testing machine that used a knife-edged loading head. The maximum load at failure was recorded and converted to mega pascals (Mpa).

Shear bond strength (Mpa) = $F (N)/\varpi r^2$

Antioxidant Activity Percentage Determination (% AA)

Diphenyl-1-Picrylhydrazyl (DPPH) free radical assay was used to assess %AA of each substance. In an ethanol solution with the stable DPPH radical samples were reacted. The reaction mixture is set by adding 60 µL of the sample to 2.960 mL of 0.5 mm DPPH solution dissolved in methanol. The blank comprised of 3 mL of methanol, and the control was 2.960mL of 0.5 mm DPPH solution dissolved in methanol and added to 60 lL of water. DPPH is lessened when reacted with an antioxidant, which can give hydrogen. The changes in color (from deep violet to light yellow) were read [Absorbance (Abs)] 517 nm after 60min of reaction, using a UV–VIS spectrophotometer. For every substance, the experiment was done in triplicate and for each average antioxidant value was calculated . According to the equation described by Mensor LL et al. %AA was determined.[15]

$\%AA = 100 [(Abs_{sample} – Abs_{branco})/Abs_{controle} \times 100]$.

Statistical Analysis

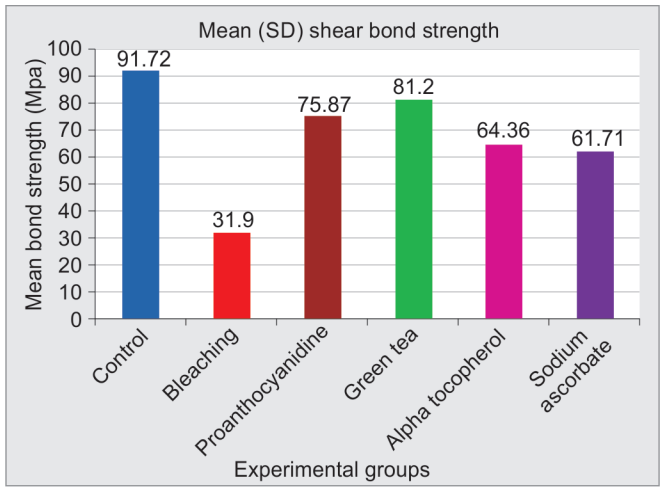
The values obtained were statistically analyzed using computer software Statistical Package for the Social Sciences (SPSS) version 16.0. One-way analysis of variance (ANOVA) and Mann Tukey Post hoc test were used to analyze the data. Significance was established at $p < 0.05$ level.

RESULTS AND OBSERVATIONS

In the present study group, A, i.e. negative control group showed significantly good bond strength in comparison to group B (positive control i.e., bleached), group C (green tea), Group D (Proanthocyanidin), group E (Alpha-tocopherol) and group F (Sodium Ascorbate). On application of antioxidants, reversal of bond strength occurred with the maximum being in group D, i.e., green tea, followed by group C, i.e., proanthocyanidin, then group E, i.e., alpha-tocopherol and least in group F, i.e., sodium ascorbate.

When group B is compared with group A, C, D, E, and F significant distinction was observed among these groups proving that group B, i.e., bleached surface showed the minimum bond strength values. On comparison of group C with group D significantly better bond strength reversal was found in group D. On comparison of group C with group E mean score values of bond strength for group C was observed to be higher. On comparison of group C with group F significantly higher shear bond strength for proanthocyanidin was seen. On comparison of group D with group E mean score values of bond strength for group D was observed to be higher than group E. On comparison of group D with group F significantly higher mean score values of shear bond strength for green tea than sodium ascorbate were observed. On comparison of group E with group F significantly higher mean score values of shear bond strength for alpha tocopherol than sodium ascorbate were observed.

Graphic representation of the comparison of mean SBS among groups is given in (Graph 1). The results of this study are shown in (Table 2). Values of DPPH assay are shown in (Table 3)



Graph 1: Mean and standard deviation of shear bond strength after using bleaching agents and antioxidants.

DISCUSSION

In the present period, patients are winding up esthetically more cognizant. Any stained tooth in the oral cavity is a state of concern. The interest for more moderate treatment is additionally increasing patient's inclination towards bleaching or veneers in contrast with broad crown arrangements. Bleaching includes an oxidative concoction that alters the light-retaining as well as light-reflecting nature of a material structure, in this way expanding its insight of whiteness. Like each system, bleaching likewise has certain drawbacks in a clinical situation . Notwithstanding the perfect results achieved with the bleaching agents, similar to carbamide peroxide and hydrogen peroxide, a couple of examinations have in like manner declared surface modifications, diminished micro-hardness, and decreased bond strength of composite resin on enamel after bleaching.[4]

Following bleaching, the traded off shear bond quality is due to bleaching agent abandoning a free remaining oxygen layer that is repressing the polymerization of composite resin and meddles with the resin penetration into the etched enamel.[11] To beat this diminished bond quality in bleached teeth, the prime requisite is to evacuate the oxygen-rich surface layer of enamel in this manner that it brings about typical bond strength.[12]

The residual peroxide and oxygen slowly dissipate

Table 3: Value of antioxidants after UV spectrophotometry using DPPH assay as a control

Test compound	*Percent inhibition of DPPH*
Proanthocyanidin	34.70
Green Tea	56.57
Sodium ascorbate	9.10
Alpha-tocopherol	16.40

Table 2: comparison of shear bond strength among various groups. There were statistically significant differences found for the shear bond strength between various groups of materials

Groups		*Mean (MPa)*	*Standard deviation*	*F value*	*Pb value*	*Tukey post hoc test*
Group A	Control	91.72	5.08	456.328		
Group B	Bleaching	31.90	3.83			A > D,C,E,F,B
Group C	Proanthocyanidin	75.87	4.50		0.000* (<0.05)	C > E,F,B
Group D	Green tea	81.2	4.64			D > C,E,F,B
Group E	Alpha-tocopherol	64.36	4.07			E > B
Group F	Sodium ascorbate	61.71	2.94			F > B

One-way ANOVA test, * Significance of relationship at $p < 0.05$

with time, eventually eliminating any reduction in composite resin bond strength associated with the bleaching. The waiting period for bonding procedures after bleaching has been reported to vary from one day to one month. In any case, this is tedious for professionals and patients. In this way, antioxidant substances have been examined with the point of speeding treatment. Antioxidants neutralize free radicals by giving one of their electrons, finishing the electron stealing reaction and change the redox capability of the blea ched surface.[11]

Consequently, n current examination and correlation of impacts of four antioxidants specifically 5% proanthocyanidin, 10% sodium ascorbate, 10% alpha tocopherol, 10% green tea on the reversal of bond quality in dyed teeth was assessed.

In the present study Group A showed significantly good bond strength in comparison to all other groups. This was as per previous investigations are done by Dabas D et al. (2016) and Kimyai S et al. (2016).[7,16] This could be due to the fact that when the enamel surface is left untreated, there is no residual oxygen and no free radicals left on the surface which occurs amid the process of bleaching. Thus, there is no effect on polymerization.[11] In any case, Manimaran et al. (2011) in their study observed that application of antioxidants enhanced the bond strength of root enamel and dentin when compared to the untreated surface which was not as per our investigation[8]. This differentiation perception can be due to different methodology and diverse substrate (root dentinal surface) and different bonding agent used.

When group B is compared with group A, C, D, E, and F significant distinction was observed among these groups proving that group B, i.e., bleached surface showed the minimum bond strength values. This was as per the investigations are done by Schwertner et al. and Kimyai et al.[9,17] The plausible explanations for compromised bond strength following bleaching is because th e bleaching agent leaves a residual oxygen layer that interferes with the resin infiltration into etched enamel and polymerization of resin is hindered, thereby affecting the bond strength.[17]

On comparison of group C with group D significantly better bond strength reversal was found in group D. The outcomes were as per past investigation was done by Shashibhushan et al. and Sharafeddin et al.[12,18] The probable explanation for the observations obtained in the present study are that the excess generation of oxygen is inhibited by green tea polyphenols, that increases the degree of polymerization and also enhances the bond strength of resin on bleached enamel. Eppigallactocatechingallate (EGCG), the most plentiful catechins in green tea, diminishes dentin demineralization by inhibiting MMPs, thereby, stabilizing the resin tooth bonding interface. Green tea additionally contains proanthocyanidin; therefore, demonstrating better oxygen scavenging ability than proanthocyanidin alone.[19] Khamverdi et al. (2016) observed better bond strength values with Proanthocyanidin in comparison to Green tea. The probable reason for disagreement is the bleaching agent used was of different concentration (40% H_2O_2) and for different application time.[14]

On comparison of Group C with Group E mean score value of bond strength for Group C was observed to be higher. No previous studies have been done to determine the comparison among proanthocyanidin and Alpha tocopherol. DPHH assay value of proanthocyanidin (34.70) is more than Alpha tocopherol (16.40). Therefore, the antioxidant potential of Proanthocyanidine is more. Thus, better bond strength reversal was seen in Proanthocyanidin group which is shown to be 20 times more potent than sodium ascorbate.

On comparison of group C with group F significantly higher shear bond strength for Proanthocyanidin was seen. The results were in agreement with previous studies done by Manoharan et al.[1] and Vidhya et al.[2] this can be due to three reasons that are:

- specificity of proanthocyanidin for hydroxyl free radicals.[20]
- The superoxide radicals being trapped by the multiple donor sites found on oligomeric proanthocyanidin (OPC).[8]
- The free radical scavenging activity enhanced by the esterification of epicatechin by gallic acid in OPCs.[1] Arumugam et al. observed that Sodium ascorbate had a better potential in elevating the bond strength of enamel surface than proanthocyanidin. The probable reasons for disagreement are the different bleaching methodology used. Also, bonding was carried out immediately after application of antioxidants.[20]

On comparison of group D with group E mean score values of bond strength for group D was observed to be higher than group E. No previous such studies have been reported till date. DPHH assay value of green tea (56.57) is more than alpha tocopherol (16.40). Therefore, the antioxidant potential of green tea is more. Hence, better bond strength reversal was seen in group D.

On comparison of Group D with group F significantly higher mean score values of shear bond strength for Green tea than Sodium ascorbate were observed. The outcomes were as per past investigations are done by Sharafeddin et al. and Ozelin et al.[21] This can be due to the presence of polyphenols and potent antioxidant activity of Green tea catechins that decreases dentin demineralization by

inhibiting MMPs, thereby, stabilizing the resin tooth bonding interface.[18,21,22]

On comparison of group E with group F significantly higher mean score values of shear bond strength for alpha tocopherol than sodium ascorbate were observed. The results were in agreement with previous studies done by Sasaki et al. and Mohan et al.[23,24] This can be due to the activity of alpha-tocopherol that terminates the chain reactions during bleaching by the exclusion of free radical intermediates which helps in inhibiting other oxidation reactions. Because of alcohol, i.e., ethanol used as a vehicle, better bond strength reversal of bleached enamel surface was seen in group E, i.e., alpha-tocopherol.[24] Also, DPHH ASSAY value for group E, i.e., alpha-tocopherol is 16.40, and for group F, i.e., sodium ascorbate is 9.10. Thus, the better antioxidant potential was exhibited by group E. Vitamin E is more oxidizing and stable than ascorbate because of its hydrophobicity.

The percentage of antioxidant activity of all the antioxidants used in our study was assessed by DPHH free radical assay using a spectrophotometer.

The radical scavenging activity of different extracts was determined by using DPPH (2, 2-diphenyl-1-picrylhydrazyl) ASSAY. The decrease in the absorption of the DPPH solution after the addition of an antioxidant was measured at 517 nm. 1, 1 Diphenyl 2- Picryl Hydrazyl is a stable (in powder form) free radical with red color which turns yellow when scavenged. It uses this character to show free radical scavenging activity. In our study the percentage inhibition of different antioxidants using UV Spectrophotometric analysis with the help of DPPH free radical assay come out to be in decreasing order as follows:

Green tea > proanthocyanidin > alpha tocopherol > sodium ascorbate

Group D > group C > group E > group F

56.57 > 34.70 > 16.40 > 9.10

Therefore further studies should be conducted to evaluate the effect of different application time, different concentrations and different types of antioxidant on the shear bond strength of bleached enamel surface with different concentration of bleaching agents.

CONCLUSION

Bleaching of enamel reduced the shear bond strength. So application of antioxidants promptly after bleaching showed significantly increased bond strength. Amongst the antioxidants tested in this study, Green tea extract was the most effective antioxidant in reversing the bond strength hence it may be an innovative option for esthetical treatment after bleaching.

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