

Influence of newer antioxidants on microtensile bond strength of composite resin to bleached enamel - An in vitro study

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Abstract

The presence of an un-aesthetic discolored tooth is of great concern to patients, which is caused by intrinsic and extrinsic factors and can be treated by bleaching, enamel microabrasion, porcelain veneers, crowns. The application of antioxidant agents after bleaching has been recommended as a way to reduce the waiting period between bleaching and restorative procedure by eliminating the reactive oxygen from the dental substrate. In our study, we found a significant improvement in the bond strength of composite to bleached enamel with the application of antioxidant agents.

Keywords: Composite, Aesthetics, Bleaching, Adhesion, Cohesion, Antioxidant.

Introduction

The presence of an un-aesthetic discolored tooth is of great concern to patients and has a profound effect on the patient's confidence and oral health, which in turn can significantly contribute to the total well-being of an individual. Tooth discoloration is caused by intrinsic and extrinsic factors, which can be treated by bleaching, enamel microabrasion, porcelain veneers, crowns.^{1,2,3}

Hydrogen peroxide (HP), an oxidizing agent diffuses into the tooth and dissociates to produce free radicals which attack the organic pigmented molecules within the tooth tissue. As a result, the chromophore molecules become smaller and less heavily pigmented and there will be a shift in the absorption spectrum, thus resulting in the bleaching of the tooth.^{2,4,5}

It has been proposed that HP penetrates enamel and dentin to reach the pulp cavity, and the residual oxygen from the bleaching agent inhibits resin polymerization. So, to prevent the deleterious effects of peroxide on the resin, a waiting period of 1-3 weeks is recommended.⁴ However, due to the immediate aesthetic requirements of the patients, other methods of reversing the reduced bond strength of enamel after bleaching becomes important.

Several studies have found no significant difference between the use of antioxidant surface treatment immediately after the bleaching procedure and delaying the restorative bonding for at least two weeks. So the application of antioxidant agents after bleaching has been recommended as a way to reduce the waiting period between bleaching and restorative procedure by eliminating the reactive oxygen from the dental substrate.^{6,7}

Enzymatic agents such as Super Oxide dismutase⁸ and non-enzymatic agents such as Sodium Ascorbate (SA), Flavonoids, Lycopene, and Vitamin E have antioxidant properties. The knowledge regarding the newer antioxidant Quercetin is limited. So, to fill the lacunae and to overcome the limitations of the previous studies we conducted this study.^{7,9,10}

Aims & Objectives

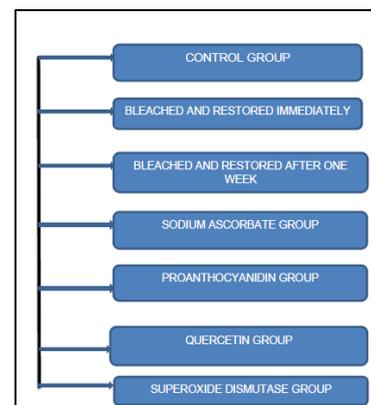
1. To compare and evaluate the micro-tensile bond strength of resin to bleached enamel using different antioxidants.
2. To determine the mode of failure of the fractured teeth.

Materials and Methods

One hundred five extracted permanent incisors without caries, cracks and fractures were obtained, cleaned of debris and stored in saline. Later, prophylaxis of the teeth was carried out and disinfected in 0.9% thymol for 24 hours.

The crowns were sectioned from the root using a low-speed rotary saw under water irrigation. The crown specimens were embedded in self-cure acrylic resin with the labial surfaces parallel to the horizontal plane. The buccal surfaces were flattened with silicon carbide sandpaper with decreasing granulations under abundant irrigation with water for 10 seconds with each sand paper and for 60 seconds with the 600 grit sandpaper, to obtain a flat surface and maintain approximately 1.5 mm of enamel remnant.

The specimens were randomly divided into one control and six experimental groups, with 15 samples in each group (n=15).



Group 1: Control group

In this group, unbleached specimens were stored in distilled water for one week and etched with Gluma etch (KULZER) for 20 sec and water sprayed to completely rinse off. The enamel surface must be dried and should appear chalky white, followed by the application of bonding agent and light-cured for 20 seconds with Bluephase N at 400-500mw/cm. A Teflon mold of (5mmx5mmx5mm) was placed approximately in the center of the labial surface and 5mm thick submicron hybrid composite (CHARISMA) was incrementally cured for 20 seconds (Figure 1). All the specimens were stored in distilled water until they were tested.

Group 2: Bleached and restored immediately

In this group, teeth were exposed to a 1mm layer of 37.5% of HP gel for 8 minutes and thoroughly cleaned with a soft micro-brush under flowing distilled water and placed in artificial saliva for remaining time of the day. A total of 5 applications were done with one-day interval. At the end of 5th day, they were restored with composite similar to that of the control group protocol and stored in distilled water.

Group 3: Bleached and restored after one week

In this group, teeth were bleached similar to the protocol which was followed in group-2 and stored in distilled water for one week, later composite build up was done following the same protocol as in control group and stored in distilled water.

Group 4: Bleached and 10% Sodium Ascorbate (SA) application

In this group, teeth were bleached similar to the protocol which was followed for Group-2. 10% SA solution was freshly prepared by adding 10gm of SA powder in 100ml of distilled water. The Solution was applied onto the bleached surface with the help of micro-brush and after 10 min the specimens were rinsed and dried and restored with composite similar to that of the control group and stored in distilled water.

Group 5: Bleached and 5% Proanthocyanidin (PA) application

In this group, teeth were bleached similar to the protocol which was followed for Group 2. 5% PA solution was freshly prepared by adding 5gm of PA powder to 100ml of distilled water. The Solution was applied for 10 minutes on bleached enamel and rinsed with a jet of water and restored with composite similar to that of the control group and stored in distilled water.

Group 6: Bleached and Super Oxide Dismutase (SOD) application

In this group, teeth were bleached similar to the protocol which was followed for Group 2. After the bleaching protocol, SOD readily available solution was applied and rinsed after 10 minutes on bleached enamel and they were restored with composite similar to that of the control group and stored in distilled water.

Group 7: Bleached and 6.5% Quercetin application

In this group, teeth were bleached similar to the protocol which was followed for Group 2. Quercetin solution was freshly prepared by adding 10gms of Quercetin powder in

10ml of Dimethylsulfoxide and 40 ml of Phosphate Buffered Saline. Solution was applied on bleached enamel and rinsed with a jet of water after 10 minutes and was restored with composite similar to that of the control group and stored in distilled water.

Micro-tensile bond test

The composite was placed on the enamel surfaces incrementally using a resin instrument to create a block of approximately 5x5x5 mm³. Each increment of 2mm composite resin was cured for 20 seconds. The bonded specimens were placed in distilled water at 37°C for 24 hours.

Subsequently, the enamel-composite bonded specimens were sectioned occluso-gingivally into slabs using hard tissue microtome. Sectioning the slabs mesiodistally generated multiple “resin-enamel-dentin” beams with a cross sectional surface area of approximately (1.2 x 1.2 x 8mm) + 0.2mm.

Molds of PVC-Poly vinyl chloride pipes were made to prepare acrylic cylinders of diameter 1 cm and height 3.5cm. Three-fourth of the cylinder was filled with self-cure acrylic in dough stage, and one end of the tooth resin rod was embedded and remaining one-fourth of the cylinder was filled self-cure polymer and monomer in sprinkle on method. Similarly the other end of the tooth-resin rod was also embedded in another acrylic cylinder as mentioned above.

Both ends of resin tooth rod were embedded in 2 acrylic blocks, such that 3mm of resin-tooth rod was exposed, which consisted of 1.5mm of enamel and 1.5mm of resin. The tooth-resin rod was secured with a cyano-acrylate resin to a modified testing jig attached to UTM-Universal testing machine. The specimens were stressed in tension mode at a crosshead speed of 0.5mm per minute until failure. The failure strength was determined as the quotient of Load on failure / Adhesive area (A) and was recorded in MPa.

Results

In this study, the bond strength of composite to bleached enamel was evaluated using the following antioxidants Sodium Ascorbate, Proanthocyanidin, Superoxide dismutase, Quercetin.

The statistical analysis was done by using ANOVA test and comparative statistical analysis was done by using Dunnett's test and Tukey test.

Analysis of Variance (ANOVA) was used to determine the test differences between means of study groups. Tukey test was used to determine intergroup difference between 3 or more experimental groups other than control group. (Table1 & 2)

Under the experimental conditions of the current in vitro study, the control group and the experimental group Quercetin showed the highest micro-tensile bond strength, and group 2 i.e.,” immediate bonding after bleaching” group showed the lowest micro-tensile bond strength. All the data were subjected to normality test (Shapiro-Wilk test) and the data followed normal distribution. Statistical analysis was

performed using SPSS21 software. Overall significance of micro-tensile bond strength within each group was evaluated using one way ANOVA test.

The mean micro-tensile bond strength values are highest for group-1 (16.31 MPa) and least for group-2 (5.03MPa). The mean values are in descending order group-1 followed by group-7, group-4 and group-3, group-5, group-6, group-2. (Table 1).

There was no statistical difference in micro-tensile bond strengths between group-1, group-3, group-4, group-5 and group-7. There was a statistical significant difference between group-1, group-2 and group-6. (Table-2).

The results of stereomicroscopic examination revealed three types of failure modes adhesive (occurred purely at the restorative/dentin interface), cohesive (occurring purely within the material or purely within dentin), and mixed (combination of both adhesive and cohesive mode). Adhesive failure indicates that the failure occurred due to lack of sufficient bond strength of the composite material to the tooth. A cohesive failure on the other hand, indicates that the bond strength was greater than the strength of composite materials used. A mixed failure generally indicates when the bond strength and strength of the supporting composite materials were close enough to each other to exhibit both failure modes.

In the present study, group-1(control) showed 40% adhesive failures, 20%cohesive failures, 40% mixed failures. Group-2(immediate bonding after bleaching) showed 60% adhesive failures, 13% cohesive failures, 27% mixed failures. Group-3 (delayed bonding of bleached enamel) showed 47% adhesive failures, 27% cohesive failures, 40% mixed failures. Group-4 (SA) showed 33% adhesive failures, 40%cohesivefailures, 27% mixed failures. Group-5 (PA) showed 33% adhesive failures, 27% cohesive failures, 40% mixed failures. Group-6(SOD) showed 40% adhesive failures, 13%cohesive failures, 47% mixed failures. Group-7 (Quercetin) showed 40% adhesive failures, 47% cohesive failures, 27% mixed failures (Fig. 2).

Adhesive failure occurred purely at the restorative/dentin interface resulting in reduced bond strength between resin and dentin which is unfavorable. In the present study, cohesive failure within the restorative material exhibited by

group-7(Quercetin) and group-4(SA) are considered favorable compared to group-6,5and 2 which showed more mixed failures and is least desirable in the clinical conditions (Fig. 2).

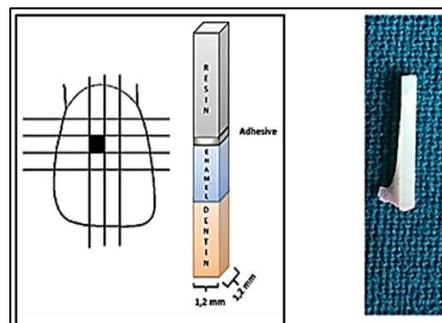


Fig. 1a: Enamel resin rod

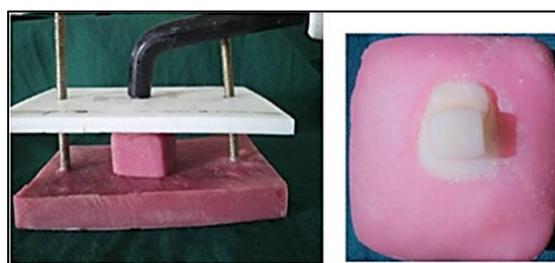


Fig. 1b: Composite restoration with teflon moulds

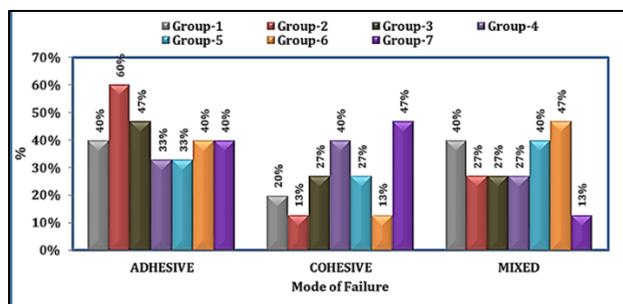


Fig. 2: Mode of failures of all the groups

Table 1: The mean and standard deviation values and confidence intervals of microtensile bond strength in (MPa)

Group	N	Mean	SD	95% Confidence interval for mean	
				Lower bound	Upper Bound
Group-1	15	16.31	4.42	13.86	18.76
Group-2	15	5.03	1.43	4.24	5.82
Group-3	15	15.34	5.57	12.25	18.43
Group-4	15	15.24	4.55	13.02	18.06
Group-5	15	12.37	7.02	8.48	16.25
Group-6	15	6.09	1.77	5.11	7.07
Group-7	15	16.01	5.18	13.14	18.88

F=16.17; P<0.001

Table 2: Inter group micro tensile bond strength

	G1	G2	G3	G4	G5	G6	G7
G1		<0.001	.998	.999	.249	<0.001	1.000
G2	<0.001		<0.001	<0.001	0.001	.996	<0.001
G3	.998	<0.001		1.000	.589	<0.001	1.000
G4	.999	<0.001	1.000		.510	<0.001	1.000
G5	.249	.001	.589	.510		.007	.340
G6	<0.001	.996	<0.001	<0.001	.007		<0.001
G7	1.000	<0.001	1.000	1.000	.340	<0.001	
Test applied Dunnet test and ANOVA P<0.05- significant difference							

Discussion

There are three fundamental approaches for bleaching of vital teeth; in-office or power bleaching; at home or dentist-supervised night-guard bleaching; and bleaching with over-the-counter (OTC) products. In-office bleaching utilizes a high concentration of tooth-whitening gels (25-40% HP) and different types of curing lights like Halogen curing light; Plasma arc lamps; Xe-halogen light; Diode lasers; or metal halides to activate the bleaching gel.⁴

Post-operative sensitivity is one of the most common complications of bleaching, which accounts for 62.2%.¹² Clinical researches reported a prevalence of 18-78% of sensitivity either with at-home tray delivery or in office procedures.^{4,13,14}

Another complication of bleaching is decreased bond strength of composite resin to enamel. The decrease in the bond strength is significant when the composite restoration is performed immediately after bleaching. It was observed that bleaching may increase the solubility of glass-ionomer and other cements. The bond strength between enamel and resin based fillings was reduced by 40-55% when bonded immediately to bleached enamel.^{4,5,11} The most common reason for decreased bond strength of resin to bleached enamel is the oxidative effect of HP.

Various techniques have been recommended to counteract the impaired resin-adhesion problem. Commonly recommended solution is delaying the restorative treatment by 1-3 weeks to allow the degradation of residual oxygen.¹⁵ According to some authors, the recommended waiting period for restorative procedures on enamel after bleaching with 10% CP is 24 hours.¹⁶ Some authors suggested a waiting period of one week.¹⁷ Many authors claimed that a period of 24 hours is not enough to re-establish the bond strength to enamel for higher concentrations like 9.5, 25,30,35 and 38% HP and at least 1-3 weeks is necessary.^{6,7,15}

Clinically this can be a long period for patients seeking cosmetic treatment, so restorations were done immediately after bleaching to satisfy patient's aesthetic needs. So, the application of various anti-oxidants was advocated to allow immediate bonding without compromise in the bond strength, which was proved by various researchers, though their degree of regaining bond strength was not uniform.^{18,19,20,21}

Removal of superficial bleached enamel (0.5-1mm) was another method used to increase the bond strength. This can be achieved by the flattening of the enamel surface after bleaching with the help of diamond points.¹⁵ The drawback of this method is that with the increase in the aesthetic demands and minimally invasive procedures, this method cannot be applied to the clinical situation like no preparation veneers, diastema closures where there is absolutely no preparation of tooth.¹⁵

Apart from the above mentioned methods, another technique is use of adhesives containing organic solvents.²² Pre-treatment of bleached enamel with alcohol decreases the residual water and oxygen; and increases the bond strength of composite resin to bleached enamel.⁷

Among the various anti-oxidants used, Sodium Ascorbate is the most commonly used antioxidant which became the standard of care. Another antioxidant is α -Tocopherol, which is the most active component of vitamin E Complex. Quercetin is one of the flavonoids with antioxidant property. It is available in fruits and vegetables.

Various storage medium can be used to store the testing specimens like distilled water, saline, artificial saliva or formaldehyde solution.²³ Saline was used in our study to store the teeth after extraction, surface preparation and after specimen preparation as other solutions were reported to effect the resultant bond strength. The teeth used for in vitro bonding studies are mainly obtained from humans and bovines.

In the present study, a crosshead speed of 0.5mm/min was chosen as no clear advantage or relationship of scatter of data was observed using lower cross head speeds to debond the specimens.

Under the experimental conditions of the present study the results demonstrate that the mean micro-tensile bond strength value of group I (Control) is higher than the experimental groups with no statistical difference between control group and Quercetin (Table 1). This was in accordance with the study done by Mana Shamsed in et al. who observed that these experimental concentrations of Quercetin were capable of improving the SBS to normal levels.

In our study, the mean micro-tensile bond strength of group 2 was least when compared to those of control and experimental groups with a statistically significant

difference. There is 69% decrease in bond strength when compared to group 1 (Table 1).

This is in accordance with the studies done by Amal Suleiman et al, Fahim et al, Moosavi et al. who observed that peroxides and their by-products present inside the dental structure are capable to interfere with the polymerization process of the adhesive material there by decreasing the bond strength.

In the present study the mean micro-tensile bond strength of group 3 was 5.8% lower than the control group and there was no statistical difference between group 3 and 4. There was statistical difference present between group 3 and 2 (Table 1). These were in accordance to the previous studies Murad et al, Mehdi et al, Hamid et al. They concluded that the anti-oxidizing ability of SA helped to neutralize and reverse the oxidizing effects of the bleaching agent. Therefore, the altered redox potential of the oxidized bonding substrate is restored and polymerization of the adhesive continues without permanent termination. The results of our study support these findings.

In the present study there was no significant difference in the mean micro-tensile bond strength values of group 4 (SA) and group 5 (PA) which was in accordance with the previous studies. Zahra et al, Vidya et al, Manoharan et al, observed that the specificity of OPC for hydroxyl free radicals and the presence of multiple donor sites on OPC trap superoxide radicals. The antioxidant ability of Sodium Ascorbate helps to neutralize and reverse the oxidizing effects of bleaching agents.

In the present study, the mean micro-tensile bond strength values of group 6 (SOD) were less than group 5 (PA) (Table 1). This was in contradictory to the study done by Megha Nair et al, who observed that the application of SOD to the bleached enamel produced higher μ TBS values than the application of PA. However, there are very few studies and clinical research data was available.

In the present study, comparing the control group with the experimental groups, there was 69% reduction of bond strength values of group 2 compared to that of control group. There was a difference of 5.8% in mean μ TBS values between group 3 and 1, with group 1 higher than that of group 3.

Conclusion

We conclude that a significant improvement in the bond strength of composite to bleached enamel was seen with the application of antioxidant agents. Further, among the experimental groups Quercetin application showed highest mean bond strength values when compared to others. Application of Quercetin resulted in bond strength nearly equal to the control group. In terms of mode of failure, Quercetin application group showed more favorable kind of failure than other groups.

Source of funding

None.

Conflict of interest

None.

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