

Comparative evaluation of superoxide dismutase, alpha-tocopherol, and 10% sodium ascorbate on reversal of shear bond strength of bleached enamel: An *in vitro* study

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ABSTRACT

Objective: The aim of this study was to investigate the neutralizer effect of antioxidant agents on the bond strength of bleached enamel. **Materials and Methods:** Sixty enamel slabs were prepared from 60 freshly extracted maxillary central incisors and were divided into six groups. The negative control group received no bleaching treatment and the other groups were bleached with 35% carbamide peroxide (Opalescence Quick; Ultradent, South Jordan, USA). In Group II, composite was built immediately after bleaching and cured without any antioxidants. In Group III, bleached specimens received composite build ups delayed by 1 week. In Groups IV, V, and VI bleached specimens received applications of superoxide dismutase (SOD), sodium ascorbate (SA), and tocopherol solutions, respectively, for 10 min. Following composite bonding, the micro shear bond strength (μ SBS) was measured at a speed of 1 mm/min in universal testing machine. **Statistical Analysis Used:** The μ SBS values of all the groups were analyzed using the analysis of variance followed by Tukey honestly significant difference *post-hoc* test. **Results:** Bonding of composites to unbleached group (Group I) exhibited the highest mean SBS values and among the antioxidant-treated groups, the highest SBS values were seen with SOD (Group IV) treated samples (23.0040 ± 4.30565 MPa). **Conclusions:** Application of SA, alpha-tocopherol, and SOD can effectively reverse the bond strength with bleached enamel. SOD gave a comparatively more promising reversal of bond strength than SA and alpha-tocopherol, and deserves further studies.

Key words: Alpha-tocopherol, shear bond strength, sodium ascorbate, superoxide dismutase

INTRODUCTION

The introduction of newer bleaching materials in the recent years has witnessed impressive esthetic outcomes clinically. However, an additional restorative treatment is often needed to attain the desired esthetics, even after bleaching.^[1] The adverse effects of bleaching dental enamel are of considerable clinical relevance when bonded composite restorations ensue bleaching treatment. An important consequence of the use of carbamide peroxide is decreased bond strength of the composite resin to enamel immediately after bleaching procedure.^[2,3] This decreased bond strength has been ascribed to the presence of oxygen ions that inhibit resin polymerization.^[4-6] The reduced shear bond strength (SBS) might also be

attributable to alterations in the microstructure of bleached enamel surfaces after becoming acid-etched, including reduced micro hardness, calcium loss, over etching, and loss of enamel prisms.^[7] A delay by around 1–3 weeks in composite restorations has been

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suggested to overcome this. However, application of antioxidants such as sodium ascorbate (SA) and alpha-tocopherol can be implemented to avoid this delay.^[8] Bulut *et al.*^[9,10] in 2005 and 2006 studied the efficacy of delayed bonding and antioxidant application and reported that both approaches were effective in returning the compromised bond strength back to the control levels. Antioxidants neutralize free radicals by donating one of their electrons, ending the electron stealing reaction, and restoring the altered redox potential of the bleached surface.^[8] Therefore, restorative procedures can be carried out without delay after bleaching, which shortens the overall time needed for esthetic procedures.

SA has been used in various concentrations and durations to improve composite bond strength after bleaching. Ten minute application of SA in solution form has yielded favorable results.^[8] Super oxide dismutase is an antioxidant as well as a free radical scavenger enzyme. It catalyzes the dismutation of super oxide radicals to hydrogen peroxide and molecular oxygen and plays a crucial role in the protection of cells against the harmful effects of oxygen radicals.^[11] Vitamin E (alpha-tocopherol), a natural and powerful antioxidant achieves reversal of bond strength by scavenging free radicals and molecular oxygen, a mode of action very similar to ascorbic acid. The beneficial effect of alpha-tocopherol is attributed to its antioxidant and alcohol solvent effect. It has recently been suggested for improving composite bonding after bleaching.^[12]

SBS tests have been widely preferred to determine the bonding ability of composite because of their technical simplicity in contrast to tensile bond strength test. More recently, a new test method using specimens with reduced dimensions has been advocated by some authors to evaluate the SBS, as a substitute for lower trial shear for the so-called microbond "or" micro SBS (μ SBS) test. The aim of the study is to determine the bonding between carbamide peroxide bleached enamel and composite by μ SBS testing and to evaluate the reversal of bond strength after bleaching using various antioxidants (10% SA, super oxide dismutase, and alpha-tocopherol).

MATERIALS AND METHODS

The study was approved by the Institutional Ethical Committee. Sixty human single-rooted maxillary central incisors freshly extracted due to periodontal reasons, which were free of caries, crack, fractures,

or other defects, were collected. Teeth were cleaned with ultrasonic scaler and stored in distilled water until usage.

Specimen preparation

The crowns of the teeth were separated at the cemento-enamel junction using a diamond disk. Sixty enamel slabs measuring 3 mm \times 3 mm \times 2 mm were prepared from the crowns of the teeth using a two-sided diamond disk (Brasseler Savannah, USA) under water spray. Then, the enamel specimens were mounted in self-curing acrylic resin and polished using Soflex disks (3M ESPE, MN, USA) under water spray to produce flat surfaces. The specimens were then randomly divided into six groups of 10 specimens each.

Ten specimens were randomly selected for the negative control group. They were stored in distilled water at 37°C for 3 weeks and received no bleaching treatment (Group I-control group); the water was changed every day.

All the other groups were subjected to bleaching treatment which were exposed to a predetermined volume (0.01 mL) of the bleaching agent containing 35% carbamide peroxide (Opalescence Quick; Ultradent, South Jordan, USA) and applied over the prepared specimens using microbrushes.

In Group II, the surface of enamel specimens was exposed to a predetermined volume (0.01 mL) of the bleaching agent containing 35% carbamide peroxide (Opalescence Quick; Ultradent, South Jordan, USA) for 30 min. During the intervening period after rinsing the bleaching agent under running water for 1 min, the specimens were kept in distilled water at 37°C. Immediately after bleaching, etching, bonding, and curing with resin composite (Filtek P-60, 3M/ESPE) were done, without any antioxidant application.

In Group III, the surface of enamel specimens was exposed to a predetermined volume (0.01 mL) of the bleaching agent containing 35% carbamide peroxide (Opalescence Quick; Ultradent, South Jordan, USA) for 30 min. During the intervening period after rinsing the bleaching agent under running water for 1 min, the specimens were kept in distilled water at 37°C. Etching bonding and curing with resin composite (Filtek P-60, 3M/ESPE) were performed after 1 week, without antioxidant application.

In Group IV, subsequent to the application of 35% carbamide peroxide similar to Group II, the surface

of the enamel specimens was exposed to superoxide dismutase (SOD) (Sigma Aldrich, USA) for 10 min. The aqueous solution of SOD was obtained in sodium sulfate solution. Composite build up was done and cured immediately.

In Group V, subsequent to the application of 35% carbamide peroxide, the surface of the enamel specimens was exposed to 10% SA for 10 min. During this exposure, the solution on the enamel surface was agitated every 10 min using a sterile brush. After the application of SA, the enamel surfaces were thoroughly rinsed under running water for 30 s and then kept in distilled water at 37°C. The 10% SA solution was prepared by dissolving 10 g of SA powder (TAICA Lab, Pondicherry, India) in 100 ml of distilled water in a standard flask. Composite was built and cured immediately.

In Group VI, subsequent to the application of 35% carbamide peroxide, the surface of the enamel specimens was exposed to tocopherol solution for 10 min. The solution was prepared by dissolving 10 g of alpha-tocopherol gel (TAICA Lab, Pondicherry, India) in 100 ml of ethyl alcohol in a standard flask. Composite resin was built and cured immediately.

Standardization of bonding surface and composite placement

Prefabricated polytetrafluoroethylene (PTFE) sheet mold with an iris of 0.7 mm internal diameter and 1 mm thickness was placed on the prepared buccal surfaces in all the specimens with the help of double side adhesive sticker to get a standardized bonding surface of 0.7 mm diameter on enamel before bonding procedure. Composite (Filtek P-60, 3M/ESPE) build-up of 0.7 mm diameter and 1 mm height was done in all the groups as follows.

Etching of the prepared specimens was done with 34% phosphoric acid (Dentsply Caulk, Dentsply Int. Inc., Milford, DE, USA) for 15 s, rinsed for 30 s, and then air dried for 20 s. Magic Bond (Vigodent, Rio de Janeiro, RJ, Brazil) resin adhesive was applied in a thin layer over the etched enamel using microbrushes and gently air spread. Magic bond adhesive is composed of hydrophobic monomers (BisGMA and methacrylic acid ester), without the presence of a solvent. The light cured composite resin (Z250-3M, St Paul, MN, USA) was packed into the opening of the PTFE sheet mold of dimension 0.7 mm in diameter and 1 mm in height using a Teflon-coated instrument and light cured for 40 s. Following curing, the mold

was removed and additional curing was done for 40 s from opposite sides. This insures optimal resin polymerization and homogeneous stress distribution to the tooth resin bonding interface when performing the shear test. The resulting cylindrical test specimens are of the dimension of 0.7 mm diameter and 1 mm in height. The specimens were accordingly identified and stored in distilled water at 37°C for 24 h prior to testing.

Shear bond strength evaluation

Each specimen with acrylic mold was attached to jig of universal testing machine. A thin orthodontic ligature wire of 0.2 mm diameter was embedded in 1 cm × 1 cm acrylic resin block and suspended from the holding jig and looped around the composite cylinder and gently held flush against the enamel composite interface and tested. The wire loop and the center of load cell were aligned along a straight line to ensure correct direction of application of the shear force. Shear force was applied in a universal testing machine (Instron 3369, UKAS Corporation, University Ave, Norwood, MA 02062-2643, USA) operated at a cross head speed of 1 mm/min².

Statistical analysis

Statistical analysis for the calculated means and standard deviation of the μ SBS values of the six groups was done using SPSS version 20 (IBM, Corp, USA) using the one-way analysis of variance (ANOVA) test. Tukey honestly significant difference *post-hoc* test was done to ascertain any significant differences between groups ($P < 0.05$).

RESULTS

The bond strength values among the various groups differed significantly from each other. It was observed that about 95% of failures were adhesive in nature. The control group (Group I) exhibited the highest mean bond strength value (23.004 MPa), closely followed by the SOD group (Group IV) (22.104 MPa). The least values were exhibited by the immediate bonding group (Group II) [Table 1]. The mean μ SBS values are represented in the bar graph in Figure 1.

One-way ANOVA test [Table 1] revealed that mean μ SBS value of Group IV differed significantly from the mean μ SBS values of other test groups. Direct comparisons between the groups revealed statistically significant discrepancies in the bond strength values among the six groups and that the SBS value of Group IV was significantly higher than all the other

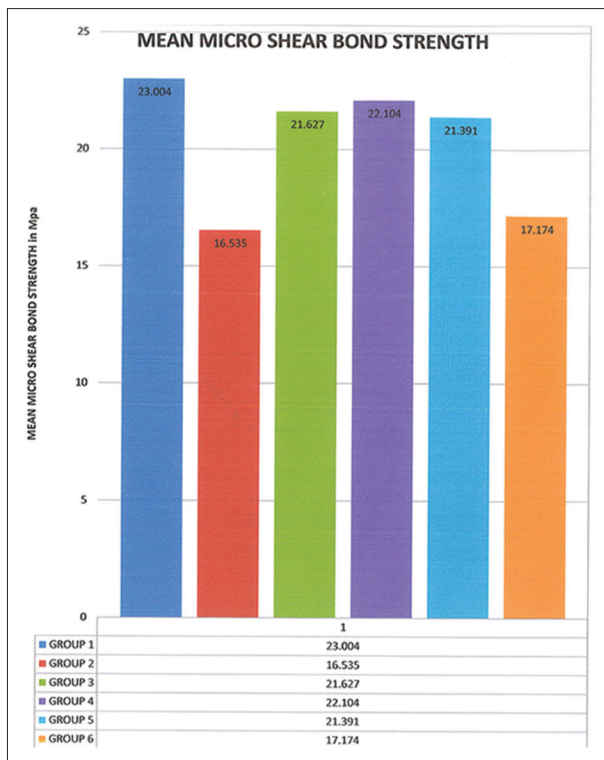


Figure 1: Bar graph showing the mean micro shear bond strengths of various groups (MPa) (original)

test groups. The μ SBS value among Groups I, II, III, IV, V, and VI was statistically significant at $P < 0.001$ [Table 2].

DISCUSSION

A significant decrease in the bond strength of composite resins has been reported after using carbamide peroxide when compared to unbleached enamel.^[13,14] The results of our study were also consistent with previous studies in terms of bond strength of bleached enamel as seen in Group II. Cavalli *et al.*^[15] reported that various concentrations of carbamide peroxide (i.e., 10%, 16%, and 20%) affect the bond strength of composite to enamel. Akin *et al.*^[16] assessed the effect of bleaching agents on the SBS of orthodontic brackets and concluded that 38% hydrogen peroxide adversely affected the bond strength of the orthodontic brackets. The lower bond strengths of bleached enamel and dentin are due to the imbalance in the redox potential caused by the bleaching agents.^[17-21] Some authors postulate that the oxygen endures in the dental structure after bleaching and can impede with the polymerization of adhesive monomers.^[20,22] Factors such as loss of calcium, decrease in micro hardness, and alterations in the organic part of the substrate

Table 1: Values of mean and standard deviation of micro shear bond strength of the control and test groups

Groups	Micro shear bond strength (mean \pm SD)	P
I	23.00 \pm 4.31	<0.001
II	16.54 \pm 3.31	
III	21.63 \pm 5.19	
IV	22.10 \pm 4.06	
V	21.39 \pm 3.46	
VI	17.17 \pm 3.69	

SD: Standard deviation

Table 2: One-way analysis of variance comparison within and between the groups

Groups	The P values of the <i>post-hoc</i> test					
	I	II	III	IV	V	VI
I	-	0.021*	0.971	0.001*	0.943	0.008*
II	0.021*	-	0.141	0.001*	0.185	0.999
III	0.971	0.141	-	0.001*	10.00	0.063
IV	0.001*	0.001*	0.001*	-	0.001*	0.001*
V	0.943	0.185	10.000	0.001*	-	0.086
VI	0.008*	0.999	0.063	0.001*	0.086	-

*Statistically significant

might be responsible for a decrease in enamel bond strengths.^[23] The residual oxygen from the bleaching agent interferes with resin polymerization.^[6] Free radicals contain unshared electrons which are highly energetic and react rapidly with oxygen to form reactive oxygen species (ROS). Previously various antioxidants have been tried for reversal of bond strength including ascorbic acid, SA, alpha-tocopherol, proanthocyanidins, pinebark extract, and catalases. Recently, Han *et al.* evaluated the effects of antioxidants after bleaching and its influence on the microleakage of composite restorations and concluded that catalase was more effective in reducing microleakage associated with reduced bond strength.^[24] In this study, we used various antioxidants for the evaluation of reversal of bond strength of enamel. Vitamin E, Vitamin C, and SOD are natural antioxidants which can deactivate free radicals.

SA is a cost-effective water soluble and commonly available antioxidant material. Many authors have evaluated its effect on the bleached teeth when composite resins have been used as bonding adhesives.^[3,12,25] In 2002, Lai *et al.*^[21] asserted that when an antioxidant such as SA was applied for 3 h to enamel after bleaching with carbamide per-oxide, the composite SBS was improved.^[9,21] Kunt *et al.*^[26]

and Lai *et al.*^[21] suggested that SA allows free radical polymerization of the adhesive resin to proceed without premature termination by restoring the altered redox potential of the oxidized bonding substrate and hence reverses the compromised bonding. In 2011, Lima *et al.*^[27] showed that even short durations of 10% SA application (i.e. 1 min) could still obviate the detrimental effect of bleaching on SBS. According to Suneetha *et al.* in 2014,^[28] 10% SA solution was effective in the reversal of SBS immediately after bleaching. Subramonian *et al.*^[29] in 2015 showed that application of 10% SA immediately after bleaching could neutralize the residual oxygen and could reverse the reduced bond strength, but scanning electron microscope images have demonstrated that application of ascorbic acid resulted in super etching of the already bleached enamel surface.^[30]

SODs are a class of metal co-factored enzymes discovered by Irwin Fridovich and Joe McCord that detoxify these free radicals by catalyzing the dismutation of superoxide into oxygen and hydrogen peroxide. SOD reduces and reverts superoxide-induced cell damage in the body. Free radicals cause wrinkles and precancerous cell changes in the body which is neutralized by the antioxidant and anti-inflammatory actions of SOD.^[11] SOD is of two types: Copper/zinc (Cu/Zn) SOD and manganese (Mn) SOD. Cu/Zn SOD defends the cytoplasm of the cells, and Mn SOD shields the mitochondria of the cells from free radical damage.^[11] In this study, we used (Mn) SOD as an antioxidant for reversal of bond strength.

“Vitamin E” is the collective name for a group of potent, lipid soluble, chain-breaking antioxidants. The antioxidant activity of Vitamin E could be structurally attributed to tocopherols (α , β , γ , and δ) and four tocotrienols (α , β , γ , and δ). Alpha-tocopherol is most profusely found in nature and is responsible for the reversal of Vitamin E deficiency symptoms in humans.^[31] The ROS formed as a result of fat oxidation is stopped by Vitamin E. Vitamin E acts on free radicals, which are by-products of normal metabolism and pollutants ingested by the body. These harmful chemicals damage the cells of the body and may contribute to the development of cardiovascular disease and cancer. Ten percentage alpha-tocopherol has shown high efficacy in SBS reversal of enamel and dentin submitted to a home-use bleaching treatment.^[12] In 2009, Sasaki *et al.*^[12] compared the efficacy of two different antioxidizing agents in

increasing the SBS of bleached enamel and dentin, reporting that 10% alpha-tocopherol was successful, whereas 10% SA was not. Hence, in this study, we used SA, Vitamin E, and a new antioxidant SOD for the reversal of bond strength of bleached enamel as a novel approach.

The micro shear bond test has been developed by Shimada *et al.*^[32] Advantages of micro shear bond test is that the bond test areas can be much better controlled by the use of known diameter micro bore tubing. The method Shimada employed is the application of a shear force using thin stainless steel orthodontic wire. μ SBS using 0.2 mm^[33] wire loop method was performed in this study because it is easier, very versatile test to assess the strength of the bond between aligned tissue and other dental substance. El Zohairy *et al.*^[34] demonstrated that μ SBS test appears to be more accurate in differentiating among the stronger adhesives than microtensile strength testing. Thus, in this study, we used the wire loop method for the assessment of μ SBS.

Türkün and Kaya^[3] investigated the effect of different concentrations (10%, 16%, and 20%) of carbamide peroxide on SBS of resin composite to bleached bovine enamel. They showed that all three concentrations of carbamide peroxide reduced the SBS, but higher concentrations produced greater reductions. Hence, we used 35% of carbamide peroxide which is a higher concentration used for in office bleaching. All the antioxidant solutions were applied for 10 min in this study. During the treatment period, the antioxidants SOD, alpha-tocopherol, and SA solutions were continuously refreshed, and enamel surface was agitated with sterile brush.

Results of this study showed that Group IV (SOD) and Group V (SA) have highest bond strength followed by Group III (Bond strength after 7 days) and Group VI (Vitamin E). These findings are in accordance with the previous study of Türkün and Kaya.^[3] Vitamin C also reversed the bond strength in this study which is in accordance with the previous studies.^[7,11,20,21] Vitamin E was used as a gel in this study. It also resulted in the bond strength reversal but less than in Group V (10% SA). This may be due to the nonaqueous nature of this antioxidant.

While SA and alpha-tocopherol are dietary antioxidants, SOD is a biological enzyme which acts at the very starting point of the free radical generation that is superoxide ion. At this level, the SOD nullifies

the superoxide ion by converting it into hydrogen peroxide.^[35] Also, SA and alpha-tocopherol have only a secondary antioxidant action wherein they undergo an alteration in their chemistry themselves thereby requiring the intervention of the SOD enzyme to revert them back to reduced forms.^[36] This proves SOD to be a biologically more potent and efficient antioxidant enzyme which only catalyzes the redox reaction without participating in the reaction itself, a striking property possessed by all enzymes. This property might be attributed to the higher SBS values obtained with the SOD group.

CONCLUSION

There is no study in the literature until date that has employed SOD as an antioxidant for the reversal of bond strength of bleached enamel. In the present study, SOD gave a comparatively more promising reversal of bond strength than Vitamin C and Vitamin E. SOD is a nontoxic antioxidant which has been used for the 1st time in an *in vitro* application for the reversal of bond strength. It provides newer avenues for advanced clinical research and exploring its further applications. However, further clinical studies are needed to confirm these findings.

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Conflicts of interest

There are no conflicts of interest.

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