The Effect of Two Different Antioxidants on the Shear Bond Strength and Micro-Hardness of Composite Resin to Bleached Enamel: An Ex-Vivo Study.

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ABSTRACT: Sodium ascorbate had the potential of forming a three-dimensional, porous physical scaffold that would entrap the pathogenic microorganisms like Streptococcus mutans and this adverse property affected its efficacy for enhancing the bond strength and highlighted the need for other antioxidants. polyphenols present in green tea are rapidly metabolized and show antioxidant activity. The aim of this ex-vivo study will be investigate the microhardness and shear bond strength of bleached human enamel treated with either sodium ascorbate and green tea solution. Eighty human maxillary premolar selected for this study. These 80 samples will be decoronated at the cemento-enamel junction using a slow speed water cooled diamond bladed saw and sectioned into two along the long axis. A flat enamel surface will be obtained on the buccal and lingual mid coronal enamel portion of the teeth with a 600 grit silicon carbaide paper mounted on a grinding/ polishing machine, making a total of 80 enamel surfaces available for this study. Out of 80 samples, 20 samples will not be bleached and will serve as control specimens. The remaining 60 samples will be bleached with 38% hydrogen peroxide gel according to manufacturers instruction followed by rinsing with distilled water. The 60 bleached enamel slabs will be randomly divided into 3 groups(group I, II and III) of 20 enamel slabs depending upon the type of antioxidant used. After antioxidant apply composite resin build up. Determination of KHN and shear bond strength estimation. The shear bond strength of the specimen will be tested using a universal testing machine.Bleaching procedure with 35% hydrogen peroxide adversely affects the enamel surface microhardness and shear bond strength.Both the antioxidant solution i.e. 10% sodium ascorbate and 5% green tea solution were applied on the enamel slab previously exposed to 35% hydrogen peroxide did not reversed the lost surface microhardness values when compared to normal unbleached enamel slabs, but the shear bond strength was enhanced when compared to the unbleached enamel slabs.

I. INTRODUCTION

Vital tooth bleaching generally involves application of hydrogen peroxide on the tooth surface in an office technique or application of carbamide peroxide in home technique.^{1, 2}

Carbamide peroxide and hydrogen peroxide function as oxidative agents by forming free radicals, oxygen reactive molecules, and hydrogen ions. These active molecules attack the pigments that are present in the teeth and remove them; the reason we can observe their effectiveness in whitening of the teeth.^{3, 4, 5}

In home bleaching technique, carbamide peroxide is used for 0.5-8h/day depending on its concentration under a dentist's supervision.^{3,4,5}

Bleaching treatment leaves some side effects on the teeth namely decreasing the bonding ability, causing morphological changes in enamel and dentin surface, reducing enamel wear resistance, and causing surface roughness. It also increases enamel porosity and changes the enamel and dentin mechanical features such as fracture toughness which may reduce the tooth crack resistance and strength.⁶

The shear bond strength (SBS) of composite resin bonded to the tooth surface decreases dramatically right after bleaching treatment. This reduction in SBS is related to the residual peroxides, the presence of which interfere with resin tag formation and the resin bond to the tooth, and subsequently impede the polymerization of resin monomers. The rest of oxygen disperse gradually, and after an appropriate time period (24 hours to 4 weeks), the composite resin recover its bond strength.^{7,8,9}

There are some techniques to prevent the reduction of composite resins bond strength after bleaching, such as removing superficial enamel and application of adhesives which contain organic solutions, alcohol, or antioxidants. Application of antioxidants was reported to be a reversal in the decline of SBS of composite resin to bleached enamel due to its protective role against free radical reactions.¹⁰ Sodium ascorbate, grape seed extract, green tea, pomegranate peel extract, and aloe vera are some antioxidants.

Sodium ascorbate is a neutral, nontoxic and biocompatible antioxidant that improves the bond strength of bleached enamel. The grape seed extract contains oligomeric proanthocyanidin complex which is more potent than sodium ascorbate. Antioxidant activity of dry green tea leaves is related to flanavols. Pomegranate peel extract contains effective compounds such as polyphenols whose antioxidant benefits preponderate over green tea. Likewise, the antioxidant effect of aloe vera is attributed to the polysaccharides found in the leaf gel.^{11,12}

Sasaki *et al.* showed that 10% sodium ascorbate could not reverse the oxidizing effect of 10% carbamide peroxide on enamel and could not increase the SBS.However, in another study, carbamide peroxide bleaching was followed by application of sodium ascorbate hydrogel and it was found to have increased the SBS of bleached enamel, which was proportional to the duration of application. Studies have shown that polyphenols present in green tea are rapidly metabolized and show antioxidant activity^{13, 14}. Catechins present in green tea such as epigallocatechin gallate (EGCG) are antioxidant compounds that can eliminate free radicals as well¹⁵. Sage extract also has antioxidant capacity ¹⁶. Previous studies have shown that these herbal antioxidants can reverse the decreased bond strength of composite to bleached enamel¹⁷. However, some studies have reported different efficacy values for herbal products in various application times¹⁸⁻²². Also, a large number of studies have compared only one or two herbal antioxidants with sodium ascorbate ¹⁸⁻²². Thus the purpose of this study was to evaluate the effect of sodium ascorbate and green tea on the micro hardness and shear bond strength of human enamel submitted to bleaching treatment with 35% hydrogen peroxide.

II. MATERIAL AND METHODS

The present in-vitro study of micro-hardness and shear bond strength was carried out at the Department of Conservative and Endodontics, Jaipur Dental College, Jaipur, Rajasthan, India.

Study Location: Department of Conservative and Endodontics, Jaipur Dental College, Jaipur, Rajasthan, India. **Study Duration**: November 2016 to November 2017.

Sample size: 80 teeth.

Selection method: Eighty freshly extracted intact first maxillary premolar, free of caries, cracks, fractures, restorations with non-fused and non-hypoplastic teeth were selected from extracted teeth. Human teeth used for research are to be treated as potential source of blood-borne pathogens, according to the United States Occupational Safety and Health Administration(OSHA). The centre for disease control and prevention has adopted guidelines for infection control of extracted teeth for research and teaching. Thus, the teeth were cleaned of any tissue remnants on the roots, plaque and calculus with periodontal scalers and were stored in 10% formalin for disinfection for 7 days and subsequently stored in 0.9% normal saline solution.

Inclusion criteria:

Intact maxillary premolars extracted purely for periodontal reasons

Exclusion criteria:

- 1. Teeth with presence of any type of carious lesions were discarded.
- 2. Teeth with restorations or any defects were excluded from the study.
- 3. Teeth having improper anatomy, hypoplastic and fractured teeth were discarded.

4. All teeth were inspected for the presence of cracks. Those with apparent cracks were excluded from the study.

5. Teeth with fused roots were excluded from the study.

Procedure methodology

PREPARATION OF SOLUTION-

Two solution were prepared for this study:

1. Ten grams of sodium ascorbate powder (SD Fine Chem Limited, Mumbai, India) was dissolved in 100ml of distilled water to make 10% sodium ascorbate solution.

2. Green tea (lipton, tulsinatura) was dissolved in 100ml of distilled water to make 5% green tea solution. **SPECIMEN PREPARATION-**The roots of 80 selected teeth were separated from their crowns at the cementenamel junction using a diamond disc. From the 80 selected teeth , 160 enamel slabs of 3 mm x 3 mm x 1 mm dimension were obtained by trimming the labial surfaces of the crown providing a surface area of 14 mm^2 . A stereomicroscope at 40 X magnification was employed to select the enamel slabs without any cracks or structural defects, that may compromise the results of study. The selected slabs were embedded in poly vinyl chloride molds of 2cm in diameter, by using self cure acrylic resin leaving the enamel surfaces uncovered by the resin, the slab surface was flattened with 600 grit silicon carbide paper under copious water irrigation. The prepared specimen were divided into 4groups to receive different procedure as sown in table no.1. The 60 bleached enamel slabs were randomly divided into three groups (group II, III) of 20 enamel slabs each depending upon the type of antioxidant used. Ten samples were selected for the determination of VHN and the remaining 10 samples were stored in the distilled water for 24 hrs before shear bond strength estimation.

APPLICATION OF ANTIOXIDANT-Immediately after bleaching and rinsing, the enamel slab of each specimen in group II and III was treated with sodium ascorbate and green tea solution respectively for 10 minutes and rinsed with distilled water.

Table 1. sample distribution								
Group		Antioxidant used	Type of test	Composite build up	Type of test			
Group I (N Unbleached specime	l=20) ens	None	10 for VHN	Done immediately	10 for SBS			
Group II (N Bleached specimens	N=20)	Sodium ascorbate	10 for VHN	Done immediately	10 for SBS			
Group III (N Bleached specimens	N=20)	Green tea	10 for VHN	Done immediately	10 for SBS			
Group IV (N Bleached specimens	N=20)	None	10 for VHN	Done immediately	10 for SBS			

*VHN= Vickers hardness number, SBS= shear bond strength

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 14days of bleaching and after the application of the antioxidants by three indentations performed. A 50g load was applied for 15 sec. the triplicate measurements were obtained from the surface which were averaged and expressed as one data point for each area of each specimen.

COMPOSITE RESIN BONDING PROCEDURE-The 20 samples each group I, II, III, IV of the enamel slabs, bonding agent (adaper single bond adhesive, 3M ESPE) was applied and cured as per manufactures instructions. Customized split metal casting was stabilized using a micropore tape and assembled around the samples to form a circular hole of dimensions 2mm diameter and 3mm height followed by placement of the resin composite in increments of 1mm in the hole and photo polymerization was performed for 40 sec. the metal casting was removed to obtain the samples with cylindrical extension of composite resin bonded to bleached enamel. After the removal of metal casting the prepared composite resin was again photo polymerized for 40 sec.

SHEAR BOND STRENGTH TEST-All the specimens were stored in ditilled water for 24 hrs before shear bond strength testing. The prepared samples were then subjected to shear bond strength evaluation using instron universal testing machine (Lloyd instruments ltd, UK). A knife edge shearing rod and a crosshead speed of 1mm per min was used. The load at failure was recorded by the software. The shear bond strength of the samples was calculated and expressed in megapascals (MPa) using the following equation.

STATISTICAL ANALYSIS-The data thus obtained was subjected to statistical analysis which was performed using kolmogorov-smirnov test, ANOVA, Post hoc test for multiple using Tukey test, Kruskalwallis test and chi-square test. The significance for all the statistical test was per-determined at p<0.05.

Table – 2							
Sample no.	Group I (VHN)	Group II (VHN)	Group III (VHN)	Group IV (VHN)			
1	403	350	348	300			
2	450	360	358	310			
3	440	340	338	320			
4	350	348	346	325			
5	380	345	343	315			
6	362	346	344	305			
7	365	347	345	308			
8	395	351	349	314			
9	390	339	337	325			
10	405	337	330	315			

III. RESULT

Individual values of the microhardness in VHN of all the group I to IV

Table-3							
	Micro-hardness						
	Mean	Std. Deviation	Std. Error	F- value	p-value		
Group I (Unbleached)	394.00	32.43	10.26				
Group II (Bleached and treated with Sodium ascorbate)	347.30	7.97	2.52	35.155	<0.001*		
Group III (Bleached and treated with Green Tea)	343.80	7.63	2.41				
Group IV (Bleached and non-treated)	314.00	8.33	2.63				

The effect of two different antioxidants on the shear bond stremgth and microhardness of composite...



Table-4

		Micro-hardness	
		Mean Difference	p-value
Group I (Unbleached)	Group II (Bleached and treated with Sodium ascorbate)	46.70	<0.001*
	Group III (Bleached and treated with Green Tea)	50.20	<0.001*
	Group IV (Bleached and non-treated)	80.00	< 0.001*
Group II (Bleached and treated with Sodium ascorbate)	Group III (Bleached and treated with Green Tea)	3.50	1.000
	Group IV (Bleached and non-treated)	33.30	0.001*
Group III (Bleached and treated with Green Tea)	Group IV (Bleached and non-treated)	29.80	0.003*

Table – 5							
Sample no.	Group I (mpa)	Group II (mpa)	Group III (mpa)	Group IV (mpa)			
1	4.3	3.0	1.9	1.5			
2	4.6	3.5	2.5	1.3			
3	4.5	3.4	1.3	1.6			
4	3.9	3.2	1.5	1.5			
5	3.8	3.1	2	1.7			
6	4.6	3.5	1.5	1.6			
7	4.5	3.7	1.7	1.7			
8	4.0	4.0	2.1	1.5			
9	3.6	3.9	1.5	1.4			
10	4.6	4.0	2	1.3			

Individual values of the shear bond strength in MPa of all the groups I to IV

Table-6						
	Shear bond strength					
	Mean	Std. Deviation	Std. Error	F-value	p-value	
Group I (Unbleached)	4.24	0.38	0.12			
Group II (Bleached and treated with Sodium ascorbate)	3.49	0.34	0.11	166.993	<0.001*	
Group III (Bleached and treated with Green Tea)	1.80	0.37	0.12			
Group IV (Bleached and non-treated)	1.51	0.14	0.05			

Table-7

		Shear bond strength		
		Mean Difference	p-value	
Group I (Unbleached)	Group II (Bleached and treated with Sodium ascorbate)	0.75	<0.001*	
	Group III (Bleached and treated with Green Tea)	2.44	<0.001*	
	Group IV (Bleached and non-treated)	2.73	< 0.001*	
Group II (Bleached and treated with Sodium ascorbate)	Group III (Bleached and treated with Green Tea)	1.69	<0.001*	
	Group IV (Bleached and non-treated)	1.98	< 0.001*	
Group III (Bleached and treated with Green Tea)	Group IV (Bleached and non-treated)	0.29	0.311	





Treatment of bleached teeth is challenging for dentists because they cannot immediately perform a resin restoration on bleached teeth due to the presence of oxygen or peroxide residues on the surface, since they prevent complete polymerization of adhesive resin. However, by postponing the composite restoration for two weeks following bleaching, no reduction in bond strength would occur. But, sometimes it is not possible for the patient to wait that long. Therefore, use of antioxidants like ascorbic acid or sodium ascorbate is one method to immediately increase the bond strength of composite to bleached enamel. It has been proven that application of sodium ascorbate is 1.8, which has adverse effects on tooth structure in clinical application. Short shelf life of sodium ascorbate solution or gel is another disadvantage of using it. It has been demonstrated that use of herbal antioxidants such as green tea and grape seed is an effective alternative strategy for this purpose.

Several studies have indicated the anticariogenic, antibacterial and antioxidant effects of these herbal extracts. In addition, herbal products are non-cytotoxic, easily available and affordable with long shelf life. Several studies have indicated that some of these herbal products have antioxidant properties 20 times greater than that of sodium ascorbate⁹.

Different bleaching agents are used for tooth bleaching depending on the technique. In the current study, 35% Hydrogen peroxide was used, which is commonly used for in-office bleaching. Some previous studies used other concentrations (10 to 30%) of Hydrogen peroxide.

The results of the current study showed the lowest bond strength in group 4. Also, significant differences were observed in bond strength between group 4 and other groups. It means that tooth bleaching results in a significant reduction in bond strength. The decrease in the shear bond strength of a bleached tooth is attributed to the residual peroxides which interfere with resin tag formation and the adhesion of resin to the tooth, and consequently inhibit the polymerization of resin monomers¹⁰.

Reason of this reduction can be related to production of free oxygen radicals in the process of bleaching, which can decrease the bond strength of composite to enamel. Some researchers indicated a reduction in calcium content of the enamel and a reduction in the enamel surface microhardness, which decrease bond strength while other studies confirmed that oxygen residues create porosities in the enamel surface and negatively affect the bond strength. In the current study, the results showed significant differences in bond strength between group 4 and groups 2 and 3. These results can be attributed to the antioxidant activity of green tea, sodium ascorbate. Lambert and Elias concluded that Green Tea had antioxidant activity. The antioxidant activity of Green Tea is due to its chemical formulation and polyphenolic nature. Polyphenols inhibit excess generation of reactive oxygen species, increase the degree of polymerization and enhance the bond strength of resin to bleached enamel.

However, Ozelin et al, indicated that green tea had positive antioxidant effect after 60 minutes; in shorter time periods such as 15 and 30 minutes, it did not increase the bond strength. Moreover, Berger et al, evaluated the effect of green tea as an antioxidant on bond strength of composite to tooth structure after bleaching for one and six hours and reported that green tea improved the reduced bond strength.

Oligomericproanthocyanidins are a class of polyphenolic bioflavonoids found in fruits and vegetables and are present in grape seed extract, pine bark extract, cranberries, lemon tree bark, hazel nut tree leaves, etc. They have free radical scavenging and antioxidant activity⁷. They also have antibacterial, antiviral, anti-inflammatory, antiallergic, anticarcinogenic, and vasodilatory actions.

Grape seed extract contains 98% proanthocyanidin, which is currently used as a nutritional supplement. Our study also showed the antioxidant activity of grape seed. Its possible mechanism is via the presence of donor sites on oligomeric proanthocyanidin complexes that enhance free radical scavenging and increase the antioxidative effect by esterification of polyphenols in proanthocyanidin complexes⁶. Therefore, it is capable of eliminating free radicals and can increase the bond strength of composite to bleached enamel. This finding was confirmed by Vidhya et al.

Two in-vitro studies demonstrated that grape seed extract inhibited the demineralization and enhanced the remineralization of carious root lesions.

The compromised bond strength following bleaching is due to the fact that the bleaching agent leaves behind aresidual oxygen layer which interferes with the resin infiltration into etched enamel and inhibits the polymerization of resin. During bleaching with hydrogen peroxide, peroxide apatite is formed as a result of the substitution of hydrogen radicals by peroxide ions. The structural changes caused by the incorporation of peroxide ions are eliminated upon storage for 2-3 weeks as the peroxide ions decomposes and the substituted hydroxyl radicals reenter the apatite lattice.

Sodium ascorbate is a derivative of ascorbic acid with a neutral pH. It has been reported that sodium ascorbate is a potent antioxidant capable of quenching the reactive free radicals. It neutralizes the effect on the residual oxygen layer, allows free radical polymerization of resin base materials to proceed without premature termination by restoring the altered redox potential of the oxidized bonding substrate, thus reversing the compromised bonding. So in this study, 10% solution of sodium ascorbate was used.

Studies have shown that the inclusion of peroxide ions may be reversed by the use of antioxidants. An antioxidant solution of 10% sodium ascorbate applied on the bleached enamel surface for 10 min effectively reversed the reduced bond strength. However, SEM images have demonstrated an etched appearance on enamel surfaces after ascorbic acid usage in bleached enamel specimens with the ascorbic acid causing super etching of the already bleached surface.

Another study showed that the application of grape seed extract, pomegranate peel extract, sodium ascorbate, and green tea on enamel surface bleached with 38% hydrogen peroxide neutralized the effect of residual oxygen on the bleached enamel and increased the SBS of resin composite bleaching agent, producing less residual oxygen molecules. We noticed that the effect of the antioxidant on SBS would decrease as the bleaching agent concentration decreased¹⁰.

Another study investigated the effect of 10% sodium ascorbate and the effect of delaying the bonding procedure on the SBS of resin-modified glass-ionomer (RMGI) and SBS of resin composite. The enamel surface was bleached with 9.5% hydrogen peroxide for 6 hours a day for 7 days consecutively. They reported that the SBS decreased when the tested groups were restored with resin composite immediately after bleaching; also, RMGI did not bond to bleached enamel surface immediately after bleaching procedure. Therefore, application of 10% sodium ascorbate resulted in increasing the SBS of the restorations to enamel surface after bleaching with 9.5% hydrogen peroxide¹⁶.

The utilization of plant extracts as a viable alternative to chemical and synthetic antioxidants have been encouraging. Hence in this study, emphasis was placed on the use of oligomericproanthocyanidins as antioxidants immediately following the bleaching procedure to reverse the compromised bond strength of composite resins to bleached enamel.

According to the material safety analyses, the level of health hazard for sodium ascorbate is higher than that of oligomericproanthocyanidins. Moreover, sodium ascorbate has been found to be mutagenic for mammalian somatic cells, while oligomericproanthocyanidins have no mutagenic effect when their material safety data's were examined.

However, this being an *ex-vivo* study, it cannot mimic the *in vivo* conditions. In the oral cavity, the interface between the restoration and the tooth is exposed to diverse forces that act simultaneously¹⁸. During its life time, a restoration is subjected to cyclic loading; each load is insufficient to provoke failure, but in the long-term can possibly lead to marginal deterioration and loss of restoration. Therefore, fatigue testing of dental adhesives is expected to better predict their *in vivo* performance.

Enamel surface microhardness measurements are used to evaluate changes related to the mineral content of enamel. This method has been repeatedly used to evaluate the effect of bleaching agents on tooth structure and restorative materials. A severe reduction in enamel hardness and distinct alteration in the surface morphology of bleached teeth were also reported. Different bleaching materials could produce an increase or decrease in enamel microhardness due to bleaching time.

An ex-vivo study by flaitz and hicks showed that different concentration of carbamide peroxide can remove mineral structure from enamel, causing morphological alteration with different forms and intensity and can reach to the subsurface. Crews et al verified that different bleaching agents have been known to lower the ca and p levels in human enamel. Cimilli and pameijer showed that some bleaching formulation could lower enamel hardness and cause the dissolution of calcium. In contrast, Murchison, chalrton and moore²⁰ noticed no significant changes in hardness of human enamel ex-vivo after exposure to 10% carbamide peroxide. Lee et al have also detected no influence on microhardness in bleached enamel using 10% carbamide peroxide ex-vivo condition but found an evident alteration to the enamel surface under SEM examination¹⁷.

In the current study, antioxidants were applied in the form of solution. Use of antioxidants in the form of hydrogel is recommended in future studies. In the current study, antioxidants were used for 10 minutes. Other studies are required to assess the effect of higher concentrations of antioxidants with shorter application times. Since antioxidants have a short shelf life, future studies are recommended to focus on the storage methods of antioxidants. The current study had an in-vitro design and clinical studies are needed to further elucidate this issue. Further studies should be conducted to evaluate the effect of different application time, different concentrations and different types of antioxidants on shear bond strength of bleached enamel surface with different concentrations of bleaching agents²².

The effect of bleaching agent is directly related to exposure time and concentration of the active ingredient, with an increase in exposure time and concentration of bleaching agent the oxidation process will be stronger and as a result, the effect and complication will be greater. The most common complication is weakening of enamel structure due to oxidation of organic and inorganic components and dissolution of enamel matrix, leading to loss of enamel mineral content²³.

V. CONCLUSION

Bleaching procedure with 35% hydrogen peroxide adversely affects the enamel surface microhardness and the shear bond strength. Both the allocated antioxidant solution i.e. 5% green tea solution and 10% sodium ascorbate solution when applied on the enamel slab previously exposed to 35% hydrogen peroxide did not reversed the lost surface microhardness values when compared to normal unbleached enamel slabs, but the shear bond strength was enhanced when compared to the unbleached enamel slabs. In comparison with 10% sodium ascorbate solution and 5% green tea solution was proved to be more effective in reversing the adverse effects of bleaching procedure on the enamel surface with respect to shear bond strength.

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