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Comparative evaluation of reversal of shear bond strength on bleached enamel-resin nanocomposite using antioxidants – sodium ascorbate, salicylic acid & N-acetyl cysteine: An in vitro study

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Abstract---Aim: The aim of this study was to compare the reversal of shear bond strength of bleached enamel using 3 antioxidant agents. Methods and Materials: 60 labial enamel blocks of maxillary central incisors were prepared, divided into six groups (n=10). Group I is control group without bleaching and without antioxidants, all the other groups were bleached with 30% carbamide peroxide. Group II, composite bonding done immediately without antioxidants application, Group III, composite bonding done after 7 days of storage in artificial saliva without receiving antioxidants. Group IV, bleached enamel receive sodium ascorbate (SA) antioxidant application for 10 minutes, followed with composite bonding immediately, similarly Group V and VI bleached enamel samples received salicylic acid (SAC) and N-acetylcysteine (NAC) antioxidants application for 10 minutes and composite bonding immediately. All the teeth were subjected to shear bond testing in universal testing machine. Statistical analysis used: The shear bond strength data was analyzed by ANOVA test and multiple comparisons by Tukey's post-hoc tests at a significance level of $P < 0.05$. Results: The result of this study showed that for shear bond strength, unbleached normal enamel (group I) showed highest SBS value among all the groups. Among the antioxidant treated groups high SBS value noted with sodium ascorbate (group IV), compared to salicylic acid (group V) and N-acetylcysteine (group VI). Conclusions: 1 week delayed bonding procedure and sodium ascorbate after bleaching shows a good reversal of reduced bond strength which is nearly equal to control. SAC and NAC shows promising results, but less when compared to SA.

Keywords---bleaching, shear bond strength, nano-composites, sodium ascorbate, salicylic acid, N-acetylcysteine

Introduction

Bleaching of discoloured teeth has been recognized as a conservative and safe treatment in restorative dentistry [1]. Bleached teeth may require adhesive restorations for enhancing esthetics, diastema closure or for management of caries. Composite restoration is the most commonly used adhesive restoration for anterior esthetics [2]. Hydrogen peroxide and carbamide peroxide are the commonly used bleaching agents in dentistry; both are effective in lightening discolored teeth [2]. When bonding is performed immediately after bleaching, hydrogen peroxide and carbamide peroxide (CP) bleaching agents alter the bond strength of composites to acid-etched enamel. This reduction in bond strength was found due to the presence of free radicals that interferes with resin attachment and inhibits resin polymerization [3].

Delay in bonding of 24hrs to 4 weeks is recommended following bleaching, to avoid the clinical problems related to bleaching-mediated compromised bond strength. This period is important to obtain better aesthetic results when composite resin is to be used. However, this waiting period makes it impossible to perform restorative procedures immediately after bleaching [4]. To overcome this

inconvenience, several methods have been proposed to reverse the compromised bond strength after bleaching, such as removal of superficial layer of enamel, treatment of the bleached enamel with alcohol before the restoration, use of adhesives containing organic solvents, and application of antioxidant agents, with varying degree of success.

Of these, application of antioxidant agents before bonding is the most accepted technique in the reversal of compromised bond strength after bleaching [5]. Enzymatic antioxidant agents like catalase and peroxidase, some of the non-enzymatic agents such as plant derivatives like polyphenols (ascorbic acid, tannic acid, gallic acid, salicylic acid, quercetin) and amino acid derivative like N-acetylcysteine are potent antioxidants [6]. Sparing sodium ascorbate, there is no study in the literature describing the effect of either salicylic acid or N-acetyl cysteine on the bond strength of adhesive restorations to bleached enamel. Various conventional mechanical test methods, such as shear, tensile, and flexural tests, have been used to assess dental adhesion. Tensile and shear tests are the ones most commonly used. Even though the validity of the shear test has been questioned, this test is frequently used due to its reproducible and relatively uncomplicated method [7]. Hence the purpose of this study was to evaluate the effect of antioxidant agents Sodium ascorbate, Salicylic acid, N-acetyl cysteine on reversal of the shear bond strength of composite on bleached enamel.

Method

Proper ethical clearance was obtained from the institutional ethical committee. 60 freshly extracted, human central incisors due to periodontal reasons, which were free of caries and other defects were selected. Teeth were cleaned with ultrasonic scaler and stored in 10% formalin for usage.

Specimen preparation

The individual teeth enamel was polished with prophylactic paste and labial surfaces polished with aluminium oxide abrasive paper (600 grit) to create a flat enamel surface. The crown portions of all teeth were cut down with diamond disk (Brasseler Savannah, USA) and were embedded in 60 acrylic blocks (2*4 cm) with labial surface exposed. The samples were then randomly divided into six groups of 10 specimens each and stored in distilled water at 37°C until the usage. Ten specimens were selected for control group without bleaching and without antioxidant, composite was built (2 mm diameter/1 mm height) with etching and bonding and cured in normal enamel and stored. All the other groups were subjected to bleaching treatment which were exposed to a predetermined volume (0.01 mL) of the bleaching agent containing 30% carbamide peroxide (Vivastyle, Ivoclar Vivadent, Schaan, Liechtenstein) and applied over the prepared specimens using microbrushes.

In Group – II, Bleaching was done with a predetermined volume (0.01 mL) of the bleaching agent containing 30% carbamide peroxide for 30 minutes and then rinsing the specimen under running water for 1 min for removal of bleaching agent. Immediately after bleaching, etching, bonding was done and composite was built and cured without antioxidants application. In Group – III, Bleaching was

done with a predetermined volume (0.01 mL) of the bleaching agent containing 30% carbamide peroxide for 30 minutes and then rinsing the specimen under running water for 1 min for removal of bleaching agent and specimens were stored for 1 week in artificial saliva. Preparation of artificial saliva was prepared in grams/litre according to McKnight – Hanes, Whiteford (1992) formula. After 1 week, without anti oxidants application, etching, bonding was done and composite was built and cured. In Group – IV, subsequent to the application of bleaching agent and rinsing, the prepared enamel surface of specimens were exposed to 10% sodium ascorbate (SA) for 10 minutes. After the application, the specimens were thoroughly rinsed with distilled water and then etching, rinsing and composite was built and cured immediately.

SA is water soluble. Preparation of 10% SA solution was prepared by mixing 10gm powder in 100ml distilled water. In Group –V subsequent to the application of bleaching agent and rinsing, the prepared enamel surfaces of specimens were exposed to 0.38% salicylic acid (SCA) for 10 minutes. After the application, the specimens were thoroughly rinsed with distilled water and then composite was built and cured immediately. SCA is insoluble in normal water however soluble in hot water. Preparation of 0.38% SCA Solution was prepared by mixing 380mg powder in 100ml distilled water, kept in the hot water bath and stirred well every 5 secs until the powder become completely soluble in water. In Group – VI subsequent to the application of bleaching agent and rinsing, the prepared enamel surfaces of specimens were exposed to 0.25% N-acetyl cysteine(NAC) for 10 minutes. After the application, the specimens were thoroughly rinsed with distilled water and then composite was built and cured immediately. NAC is soluble in water. Preparation of 0.25% NAC Solution was prepared by mixing 250mg powder in 100ml distilled water.

Standardization of bonding surface and composite placement [Fig-I]

Prefabricated polytetrafluoroethylene(PTFE) sheet mold with iris of 2mm internal diameter and 1mm thickness was placed on the prepared buccal surfaces of all the specimens with the help of double side adhesive sticker to get a standardized bonding surface of 2mm diameter on enamel before bonding procedure. Composite build up of 2mm diameter and 1mm height was done in all the groups as follows, Etching of the specimen was done with 37% phosphoric acid (N-Etch ivoclar vivadent, Schaan, Liechtenstein) for 15seconds, rinsed for 30seconds, and the air dried for 20seconds. Bonding was applied over the etched surface with universal bond (tetric N-Bond, ivoclar vivadent, Schaan, Liechtenstein) in thin layers with microbrush and gently air spread. The light cured composite resin(Tetric N-Ceram, ivoclar vivadent, Schaan, Liechtenstein) was packed in to the opening of PTFE sheet mold of dimension 2mm in diameter and 1 mm in height using a Teflon coated instrument and light cured for 40seconds. Following curing, the mold was removed, and additional curing was done for 40seconds this ensures better resin polymerization and better bonding with specimen and bonded specimens were immersed in distilled water at 37°C until testing.

Shear bond strength evaluation

Each specimen with acrylic mold was attached to jig of universal testing machine (Instron 5566 series, Instron, Canton, Mass., London, UK) [Fig-II]. Shear testing was performed with a mono-angled chisel, with the edge closely aligned to the bonding interface and perpendicular to the longitudinal axis of the resin composite cylinder and gently held flush against the enamel composite interface and tested. Shear force was applied by Instron universal testing machine at a cross head speed of 1mm/ min [Fig-III].

Discussion

Several studies have shown that hydrogen peroxide and carbamide peroxide based bleaching agents adversely affect the immediate bond strength of resins to enamel. There are several factors that affect the bond strength of the composite to the enamel after bleaching. Some authors have reported that residual oxygen after carbamide peroxide bleaching and its effect on polymerization of resin tags is the cause of decrease in the bond strength [8]. Some in vitro studies reported alterations in the chemical and morphological structures of enamel (Basting et al 2003, Nour et al 2006, Rodrigues et al 2001) [9-13]. Such structural alterations resulted in diminished shear strength values after dental bleaching. Poorni et al 2010 [14] indicated that bleaching agents cause changes in the levels of calcium and phosphorous those are present in the hydroxyapatite crystal, which are the main building blocks of dental hard tissue. Alqahtani MQ et al 2014 [15] stated that the changes in proteins and minerals content of superficial layers of enamel may be responsible for reduced bond strength. Others attribute compromised shear bond strength of composite to bleached enamel to resin tags in bleached enamel being less numerous, shorter and less defined than those in unbleached enamel, in addition to presence and evidence of bubbling [16]. For these reasons, they recommended that immediate bonding to bleached enamel should be avoided to allow delay in time (7 days) for the residual oxygen on the tooth surface to be eliminated Topcu et al [17].

Various studies have concluded that bleached enamel treated with antioxidant before composite bonding reversed the reduction in bond strength of composite resin, thus eliminating the need to postpone bonding. Antioxidants can delay or inhibit the oxidation of lipids or other molecules by removing the free radicals that initiate or propagate oxidizing chain reaction [18]. hence, antioxidant before composite bonding is believed to reverse the bond strength of composite resin, thus eliminating the need to postpone bonding. Various studies [19] have concluded the same. This study is aimed at evaluating the reversal of shear bond strength of enamel using various antioxidants Sodium ascorbate, Salicylic acid and N-Acetyl cysteine. Sodium ascorbate (SA) is a naturally occurring water soluble antioxidant. Greenpeppers, citrus fruits, strawberries and tomatoes are some of the sources. Ascorbic acid and its sodium, potassium, and calcium salts are commonly used as antioxidant food additives [20]. Previous studies revealed that SA used at a concentration of 10% showed good reversal of bond strength to bleached enamel [21-22]. Hence, the same concentration of SA was retained for our study as well.

Salicylic acid (SCA), a polyphenol, is a strong antioxidant, found in most vegetables, fruits, herbs and in leaves of wintergreen and willow bark. It is a key ingredient in several skin-care products for the treatment of psoriasis, acne, corns, calluses, and warts. It is also frequently used as a commercial cosmetic preservative [22]. In a study done by Sendamangalam V et al 2011 [22], polyphenols(including salicylic acid) were tested for their antimicrobial and antioxidant properties against *Streptococcus mutans* and concluded that SCA is good antioxidant at a concentration of 0.38%(3.8mg/ml). Hence we include this concentration for our study. N-Acetyl cysteine(NAC), is a pharmaceutical drug and is also a nutritional supplement used primarily as a mucolytic agent and in the treatment of paracetamol (acetaminophen) overdose[23]. It also aids in sulfate repletion and hence used in autism, where cysteine and related sulfur amino acids may be depleted. NAC, a sulfhydryl amino acid has several characteristics (scavenging of the hydroxyl radical, increased synthesis of reduced glutathione and diminished production of H₂O₂) favoring its usage as an antioxidant. NAC is also known as athiol antioxidant due to its reactivity with oxidant species like O₂, H₂O₂, and •OH [23].

NAC is a pharmaceutical antioxidant that is used in various concentrations. NAC is commercially available as 250 – 500mg tablets (0.25% - 0.5%). However, the concentration to be used on bleached enamel for reversal of shear bond strength is unknown. In a study by Rwei –Fen S.Huang *et al* 2002 [24], it was concluded that pre-treatment with NAC at a concentration of 0.1% (approx.) reduced free radical induced apoptosis. Hence, we conducted a pilot study to find out the exact conc. at which NAC has good antioxidant property for reversal of shear bond strength of bleached enamel, with normal enamel as control group. We tested NAC at a concentration of 0.1%, 0.25% and 0.5%. Of the three concentrations, 0.25% showed better results than 0.1%, with no significant difference between 0.25% and 0.5%. Hence, we selected the concentration of 0.25% for our study. All these three agents are well known for their antioxidant properties in the field of medicine and are commonly used substances and easily available. Previous studies have already concluded that sodium ascorbate(SA) shows good antioxidant property and reversal of shear bond strength of bleached enamel[25]. However, till to date, there is no published study using salicylic acid and N-acetyl cysteine for the same. Hence, as a novel approach, we used Salicylic acid and N-Acetyl cysteine as antioxidant for the reversal of bond strength of bleached enamel.

studies on the effect of different concentration of carbamide peroxide on the shear bond strength of composite resin to bleached enamel showed that higher conc. of carbamide peroxide produced greater reduction in bond strength than lower conc. Hence, we used 30%carbamide peroxide, which is the higher end of concentration for bleaching. In our study, antioxidants 10% Sodium ascorbate, 0.38% salicylic acid and 0.25% N-acetyl cysteine, were used for 10 minutes and were continuously replenished on the enamel surface using a sterile brush based on the studies where the duration of antioxidant treatment was 10 minutes. Several bond strength tests have been used to evaluate the adhesive performance of dentin adhesives. The most commonly used are the shear and tensile tests. The shear bond strength tests are more frequently used due to its reproducibility and relative uncomplicated method. A universal testing machine (Instron 5566 series,

Instron, Canton, Mass., London, UK) with a cross head speed of 1 mm/min was used. Shear testing was performed with a mono-angled chisel, with the edge closely aligned to the bonding interface and perpendicular to the longitudinal axis of the resin composite cylinder ^[26] [fig II,III].

Results of this study showed that Group III (Bond strength after 7 days) and Group IV (sodium ascorbate) have highest bond strength followed by Group V (Salicylic acid) and VI (N-acetyl cysteine). These findings are in accordance with the previous studies stated that treating the bleached enamel surface with 10% sodium ascorbate or waiting 1 week reverses the reduction of bond strength of composite. SA has higher reversal of bond strength when compared to SCA and NAC. These studies 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. SCA showed good reversal of shear bond strength next to SA. Antioxidant property of SCA is by donating hydrogen atoms from aromatic hydroxyl group to a free radical. This aromatic structure of SCA provides support to the unpaired electron. Thus it can inhibit the oxidation by removing the free radicals that initiate or propagate oxidizing chain reaction^[27] NAC also showed good reversal of bond strength, but when compared to SA and SCA it is less. As already stated, NAC is an amino acid derivative containing sulfhydryl group^[27]. The antioxidant property is mainly due to the hydrogen atom in the sulfhydryl group which can act as an electron for neutralizing free radicals. NAC can also act directly on reactive radicals. It is a powerful scavenger of HOCl and is capable of reducing HO. and H₂O₂^[28].

Statistical Analysis

Statistical analysis was done by IBM SPSS (IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp.) Mean and Standard Deviation (SD) were used to summarize the continuous data (Mean shear bond strength) [Table I]. Since the data was found to follow normal distribution (Mean > 2*SD), parametric tests were used for inferential statistics. Inter-group comparison of shear bond strength was done using the One-way ANOVA test followed by Tukey's post-hoc test for multiple pair wise (intra-group) comparison. A P-value of <0.05 was considered to be as a statistically significant difference [Table II].

Results

The bond strength values of all groups vary significantly from each other. It was observed that about 90% of failures were adhesive in nature. The control exhibited highest mean strength value (29.964 MPa) followed by group III bleaching after 1 week (28.667 MPa) which is closely followed by sodium ascorbate group (28.415 MPa) followed by Salicylic acid group (27.505 MPa) and NAC group (26.560 MPa). The least value was exhibited by immediate bonding after bleaching group (18.751 MPa) [Graph I]. One way ANOVA test followed by post hoc test were used to compare shear bond strength [Table II]. Mean shear bond strength value of group II is 37% less than group I, Mean shear bond strength value of group III is 4% less than group I, Mean shear bond strength value of group IV is 5% less

than group I, Mean shear bond strength value of group V is 8% less than group I, Mean shear bond strength value of group VI is 11% less than group I. Shear bond strength value between groups I, II, III, IV, V and VI showed that they are statistically significant at P Value < 0.001. The mean difference for shear bond strength measured for all groups, range from minimum mean value of 18.7512Mpa (bleached enamel with immediate bonding of composite) and maximum mean value of 29.9636Mpa (control -normal enamel).

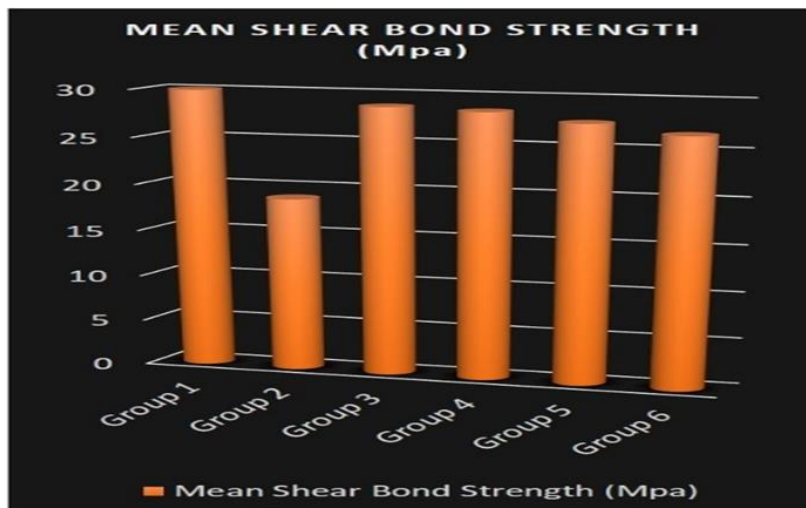
Table 1
Values of mean and standard deviation of shear bond strength (MPa) of all groups

	N	Mean	Std. deviation	Std. error	95%confidence interval for mean		Minimu m	Maximu m
					Lower bound	Upper bound		
Control (G-I)	10	29.9636	2.01903	.63847	28.5193	31.4079	26.42	33.17
Bleaching immediate(G-II)	10	18.7512	2.71327	.85801	16.8102	20.6922	13.23	21.23
Bleaching after 1week(G-III)	10	28.6672	3.04030	.96143	26.4923	30.8421	23.57	33.59
SA(G-IV)	10	28.4159	3.14819	.99554	26.1638	30.6680	22.41	32.53
SCA(G-V)	10	27.5058	2.90769	.91949	25.4257	29.5858	22.93	31.70
NAC(G-VI)	10	26.5609	2.83855	.89763	24.5303	28.5915	22.32	30.42
Total	60	26.6441	4.57874	.59111	25.4613	27.8269	13.23	33.59

Table 2
Inter-group comparison by ANOVA followed by multiple comparisons by Tukey's
post-hoc tests

Groups	The P value of the tukey's post hoc test					
	I	II	III	IV	V	VI
I	-	0.000	0.904	0.378	0.818	.089
II	0.000	-	0.000	0.000	0.000	0.000
III	0.904	0.000	-	0.938	1.000	0.550
IV	0.818	0.000	1.000	-	0.978	0.678
V	0.378	0.000	0.938	0.978	-	0.974
VI	0.089	0.000	0.550	0.974	0.678	-

*statistically significant



Graph-I. Mean Shear Bond Strength of All Groups Comparison



Fig-I. Specimen Preparation



Fig-II. Universal Testing Machine

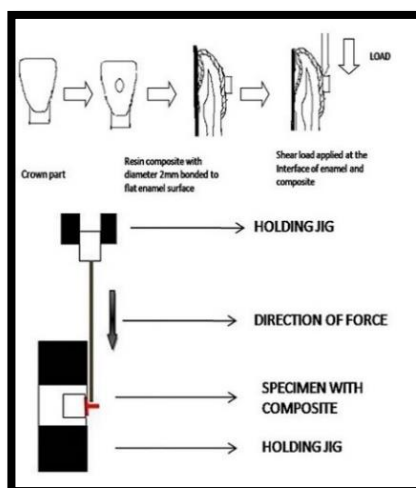


Fig-III. Diagrammatic Representation of Shear Bond Strength Test

Conclusion

Within the limitation of the present study, 1 week delayed bonding procedure and sodium ascorbate antioxidant after bleaching shows a good reversal of bond strength. Treatment of bleached enamel surface with SCA and NAC shows promising results, but less when compared to SA. Salicylic acid and N-acetyl cysteine are non- toxic antioxidants applied for the first time in in-vitro study for reversal of bond strength, gave favorable results. Hence, it provides newer avenues for further clinical research and application.

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