Original Research Paper

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"Effect of Three Vitamin Antioxidants on Reversal of Shear Bond Strength of Bleached Enamel: An In-Vitro Study"

Abstract:

Aims and objectives: To evaluate the effect of Sodium ascorbate, Alpha-tocoferol and Beta-carotene vitamins antioxidant on reversing the bond strength between resin composite and bleached enamel surface.

Materials and methods: The study focussed on use of three vitamin antioxidants. All the groups in the study were subjected to bleaching containing 35% Hydrogen peroxide. Resin restoration were done after 1 week and immediately after bleaching without any antioxidant in Group 1 and 2 respectively. While in Group 3, 4 and 5, following bleaching treatment, samples were exposed to Beta-carotene gel, Sodium Ascorbate solution and Alpha-tocoferol gel respectively for 10 minutes, before resin restoration was done. Specimens were then subjected to Universal Testing Machine. The results were statistically analysed using ANOVA test and Tukey Honest Significant Difference (HSD) test, to comparatively analyse statistical differences between the groups.

Results: The mean shear bond strength values of Group 4 (Sodium Ascorbate solution) showed significantly high bond strengths than Groups 3 (Beta-carotene gel) and 5 (Alpha-tocoferol gel).

Conclusion: Treatment of bleached enamel with Sodium ascorbate and Alpha tocoferol antioxidants has significantly improved the shear bond strength of composite resin to enamel.

Keywords: Alpha-Tocopherol, Antioxidants, Ascorbic Acid, beta Carotene

Introduction:

Esthetics is an important dimension in dental practice and is related to individuals' preferences, culture, socio demographic factors and perceived dental treatments.[1] Tooth discoloration may have a significant esthetic and social impact. In the current era of aesthetic dentistry, bleaching is readily considered for the treatment of discoloured teeth.[2] It is a treatment modality which involves an oxidative chemical that alters the light-absorbing and/or light-reflecting nature of a material structure, thereby increasing its perception of whiteness[3].

The use of hydrogen peroxide or peroxide releasing agents, such as carbamide peroxide or sodium perborate, has become a popular treatment modality[3]. Oxygen, a break down product of hydrogen peroxide, causes reduction in bond strength after bleaching as this residual oxygen interferes with resin infiltration into the dentinal tubules and inhibits resin polymerization4.Delaying the composite restorations for 1-3 weeks was recommended to overcome this problem. However, this waiting period makes it impossible to perform restorative procedures immediately after the bleaching. Several methods have been proposed to reverse the compromised bond strength after bleaching, such as:

- Removal of superficial layer of enamel5
- Treatment with alcohol before the restoration6
- Use of adhesives containing organic solvents7
- Application of antioxidant agents7

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Application of an antioxidant is commonly used as these agents restore the altered redox potential of the bleached surface and reverse the compromised bonding. They are recommended prior to bonding which allows the clinician to immediately carry out the bonding procedure.[4,8,9]

The purpose of the study was to investigate the antioxidant effect of three vitamin antioxidant i.e., Sodium ascorbate (SA), Alpha tocoferol and Beta-carotene on reversal of bond strength of composite resin on bleached enamel surface. Literature do not have much data on using vitamin antioxidants, especially Beta carotene (Vitamin A) for the reversal of bond strength. It provides newer avenues for advanced clinical research.

Material and Methods:

Hundred human single-rooted maxillary central incisors and premolars, freshly extracted due to periodontal and orthodontic reasons (free of caries, crack and fractures) were collected. They were cleaned with ultrasonic scaler and stored in distill water until usage.

The crowns of the teeth were separated at the cementoenamel junction using a diamond disk. A total of 100 enamel slabs (measuring 5 mm × 4 mm × 2 mm) were obtained, only the buccal surfaces were used. The samples were embedded in an acrylic resin block, keeping only the enamel surface exposed. Then, the samples were randomly divided into five experimental groups (n=20).

35% of Hydrogen peroxide (SDI Pola Office One Patient Bleaching Kit) was applied to enamel surface in all the groups according to manufacturer instructions and light (Blue LED) activated for 10 minutes. During the intervening period after rinsing the bleaching agent under running water for 1 minute, specimens were kept in distilled water at room temperature (37° C).

For the samples from Group1 (control group) following bleaching, etching, bonding and curing with resin composite (Filtek Z250XT, 3M/ESPE) were performed after 1 week, without antioxidant application.

For Group 2- After bleaching, etching, bonding and curing with resin composite restoration was done immediately, without antioxidant application.

For Group 3- After bleaching, specimens were exposed to 2 to 3 layers of Beta-carotene gel (Aquasol A, USV Pvt Ltd, India: Vitamin A) as an antioxidant agent for 10 minutes with the help of microbrush. Resin composite restoration was done immediately after antioxidant application.

For Group 4- After bleaching, specimens were exposed to 2 to 3 layers of 10 % Sodium ascorbate (SA) solution as an antioxidant agent for 10 minutes with the help of microbrush. Resin composite restoration was done immediately after antioxidant application.

SA solution was prepared according to a study done Kavitha et al10, by taking 2 SA tablets (Limcee 400, Abbott Healthcare Pvt Ltd, US -500mg each), finely powdered with the help of mortar pestle and then dissolved in 100 ml of distilled water in a standard flask.

Group 5- After bleaching, the enamel specimens were exposed to 2 to 3 layers of Alpha-tocoferol gel (Evion 400, Merck Ltd, India: Vitamin E) for 10min with the help of microbrush. Resin composite (Filtek Z250XT, 3M/ESPE) restoration was done immediately after antioxidant application.

Customized mylar strips (Rite-Dent, India-transparent polyester film) mold was prepared with 4 mm internal diameter and 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 4 mm diameter on enamel before bonding procedure. Resin composite (Filtek Z250XT, 3M/ESPE) build-up of 4 mm diameter and 2 mm height were done in all the groups (Figure 1) as follows.

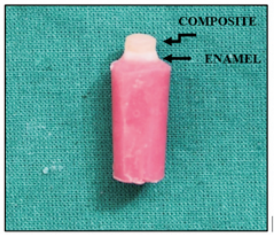

Figure1: Composite restored on the sample.

Etching of the prepared specimens was done with 34% phosphoric acid (Any Com, Any-etch 37% phosphoric acid etchant pack) for 15 s, rinsed for 30 s, and then blotted with blotting paper. Meta P & Bond (Meta-BioMed CO., LTD., Colmar, PA) resin adhesive was applied in a thin layer over the etched enamel using microbrush and cured. The light cured composite resin (3M Filtek Z250XT Composite Syringe) was packed into the opening of the customized mylar strip sheet mold of dimension 4 mm in diameter and 2 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 ensures complete resin polymerization along with homogeneous stress distribution to the tooth resin bonding interface when performing the shear test. The specimens were then stored in distilled water at 37°C for 24 h prior to testing.

The samples were subjected to shear bond shear bond strength (SBS) evaluation using Universal Testing Machine. A knife edge shearing rod and a crosshead speed of 0.5 mm per min were used. The load at failure was recorded by the software. The shear bond strength of the samples was calculated and expressed in Megapascals (MPa).

Statistical analysis for the calculated means and standard deviation of the SBS values of the five groups was done using SPSS version 26 (IBM, Corp, USA). Inter group comparison (>2 groups) was done using one way ANOVA followed by pair wise comparison using post hoc test. For all the statistical tests, $p<0.05$ was statistically significant, keeping - error at 5% and - error at 20%, thus giving a power to the study as 80%.

Results:

Among the five groups compared, Group 1 (control group) showed the highest bond strength value (71.25 MPa), followed by Group 4 (Sodium Ascorbate) (69.05 MPa), Group 5 (Alpha Tocoferol) (66.55MPa), Group 3 (Beta Carotene) (58.00MPa) and Group 2, without any antioxidant (42.60 MPa) as shown in Table 1 and [2].

Table 1: Inter group comparison of bond strength value (n=20 per group).

					95% Confidence Interval for Mean					
Group	N	Mean	Std. Deviation	Std. Error	Lower Bound	Upper Bound	Minimum	Maximum	F value	p value of one way ANOVA
1	20	71.25	11.383	2.545	65.92	76.58	50	90		
2	20	42.60	8.133	1.819	38.79	46.41	30	60		
3	20	58.00	56.48	1.263	55.36	60.64	50	68	44.776	.000**
4	20	69.05	6.337	1.417	66.08	72.02	60	80		
5	20	66.55	4.536	1.014	59.43	63.67	55	70		

Table 2: Inter group pair wise comparison of bond strength value (n=20 per group) using Post Hoc Tests.

Group (I)	Group (J)				95% Confidence Interval	
		Mean Difference (I-J)	Std. Error	Sig.	Lower Bound	Upper Bound
1	2	28.650*	2.401	.000**	21.97	35.33
1	3	13.250*	2.401	.000**	6.57	19.93
1	4	2.200	2.401	.890#	-4.48	8.88
1	5	4.700*	2.401	.001**	3.02	16.38
2	3	-15.400*	2.401	.000**	-22.08	-8.72
2	4	-26.450*	2.401	.000**	-33.13	-19.77
2	5	-23.950*	2.401	.000**	-25.63	-12.27
3	4	-11.050*	2.401	.000**	-17.73	-4.37
3	5	-8.500	2.401	.579#	-10.23	3.13
4	5	2.500*	2.401	.020*	.82	14.18

Discussion:

Despite the large number of techniques described in the literature concerning the external bleaching of vital teeth, all are based on the direct use of hydrogen peroxide (H2O2) or its precursor, carbamide peroxide.[12]

Today, most dentists use hydrogen peroxide and carbamide peroxide gels between 10-40%, chemically activated by different light sources, such as halogen light, laser or plasma arc for bleaching procedures.[13] The most common concentration of hydrogen peroxide used for bleaching purpose is 35% 12 and for carbamide peroxide it is 3% to 20% which gets decomposed into hydrogen peroxide (10% carbamide peroxide produces 3.6% hydrogen peroxide).

A significant decrease in bond strength of composite resins with tooth surface has been reported immediately after the bleaching treatment. Hydrogen peroxide is an oxidizing agent that can produce free radicals (H2O+O2), which are very reactive. The result is the perhydroxyl (HO2) is the most potent free radical. The interaction between adhesive and residual oxygen affects polymerization and results in a pattern of changes in the interface of bonding material to enamel. It also significantly decreases the micro hardness of the tooth, as stated in in vitro study by Fernandes et al in 2019.[14]

Literature demonstrates that composite resin application onto bleached enamel surfaces should be delayed at least 24 hr -1 week to restore their reduced shear bond strength 15. Clinically, however, there are situations that require adhesive restorations at the same appointment. To overcome this delay, application of antioxidants can be implemented. A study by Kaya et al[16] in 2008 stated that the application of an antioxidant after bleaching treatment improves the adhesive bond strength of bleached enamel. Various antioxidants were used in previous studies[4,17,10] such as Sodium Ascorbate, Superoxide dismutase, Butylhydroxianisole, Catalase, Ethanol, Acetone, Glutathione peroxide, Alpha-tocopherol, Sodium bicarbonate, Grape seed extract, Aloe Vera and Green tea extract, when applied after bleaching, can significantly increase the reduced bond strength after bleaching procedure.

Beta carotene (Vitamin A) comes under carotenoids antioxidant group (among 700 Crts, that are described so far).18As was communicated for the first time by Palozza and Krinsky[19], -carotene and a-tocopherol can act synergistically as an effective “radical-trapping antioxidant” in biological membranes. It is an antioxidant which has limited studies regarding the reversal of bond strength. Hence it was one of the vitamin antioxidants used in this study.

Vitamin E (alpha tocoferol) is generally considered the most important and effective lipid soluble antioxidant, maintaining cell membrane integrity from lipid and decreasing wound-healing time.4 10% alpha-tocopherol has shown high efficacy in SBS reversal of enamel and dentin submitted to a home-use bleaching treatment[20].

Sodium Ascorbate (Vitamin C) is a cost-effective water soluble and commonly available antioxidant material. Various studies[4,6,12] state that it re-establishes the altered redox potential of the oxidized bonding substrate and prevents the premature termination allowing free radical polymerization of monomers of adhesive resin.[21]

In the present study, vitamin antioxidants (used in Group 3, 4 and 5 respectively) are applied for 10 minutes on the bleached enamel surface.

The application time of 10 minutes was chosen in this study, as this duration is adequate for clinical application of the antioxidant in solution form.[8], 22For instance, the results of the study by Murad et al[23], showed that the use of 10% SA gel for 10 min is sufficient to recover bond strength. Similar study by Thapa et al4stated that 10 min application of both 10% and 25% sodium ascorbate solutions and 25% alpha-tocopherol solution may be suggested to significantly improve shear bond strength of composite resin to enamel. Also, Lai et al.[9] suggested thatthe antioxidant must be applied for at least one-third of the time of application of the oxidizing bleaching agent.

Shear bond strength assessment was done using Universal Testing Machine, as it accurately reflects the type of forces (i.e., shear forces) and magnitude of force. These are same type of forces that are generated intra orally on veneer restorations in the tooth[11].

Results of the present study are in accordance with results of following studies for sodium ascorbate (SA Vitamin C) and Alpha- Tocoferol (Vitamin E) antioxidants.

Nair-Ratihel al[24] evaluated the effect of antioxidants, namely 10% Sodiumascorbate (SA,) 10% alpha-tocopherol, 10% green tea and 10% Aloe vera on the shear bond strength of composite resin to enamel following extra-coronal bleaching using 40% hydrogen peroxide. Result of the study suggested that among antioxidants, SA (18 MPa) and alpha tocopherol (17 MPa) produced non-significantly greater shear bond strength compared to control group (18.23 MPa). These results were similar to the values of shear bond strength

the present study which are 69.07 MPa, 61.55 MPa and 71.25 MPa for SA, alpha tocoferol and control group respectively.

Study by Turkun et al[25] statedthat treatment of the bleached enamel surface with SA reverses the reduced bond strength (22.80 MPa) when compared to group where resin restoration was done immediately after the bleaching treatment (16 MPa) and may be used an alternative to delayed bonding. The results of this study are similar to the present study in which SA has shown significant reversal in bond strength (69.05 MPa) compared to samples which were restored just after the bleaching treatment (42.60 MPa). Another study conducted in 2014 by Arumugam et al,[23] where antioxidants 10% SA, 6.5% proanthocyanidin, and 5% lycopene were used, concluded that among the antioxidant groups, SA (Vitamin C) showed significantly higher bond strength reversal, compared to proanthocyanidin and lycopene.

Therefore, from the present study it can be inferred that the Control group i.e., Group 1 in which the restoration was delayed for one week, showed the highest mean bond strength value (71.25 MPa) and lowest was shown by Group 2 (42.60 MPa) where the restoration was done immediately after the bleaching procedure. These findings are in accordance with the previous studies [4,7,25,16].

Among vitamin antioxidants used in the study, Sodium Ascorbate (Group 4) has the highest bond strength value (69.05 MPa) followed by Alpha tocoferol (Group 5) (66.55 MPa), which are in accordance with the previous studies[4,6,24,23] and least (56.48 MPa) for Beta carotene (Group 3). As of now, the reason for the lowest bong strength value with Beta carotene among other vitamins cannot be assessed, therefore, further research is required.

Conclusion:

Within the limitations of this study, it can be concluded that use of bleaching agent (35% hydrogen peroxide) could significantly affect the micro-shear bond strength between the restoration and dental substrates. The reduction in bond strength has been shown to be reversible and the best method according to the literature, is to postpone the bonding procedure for 24 h upto 4 weeks after bleaching. The application of vitamins i.e., Sodium Ascorbate solutions and alpha-tocopherol solution for 10 min has been suggested to significantly improve the shear bond strength of composite resin to enamel.

Further studies need to be conducted using higher concentrations of antioxidants so that complete reversal of reduced bond strength can be achieved. Also, study did not evaluate whether the bond failure is cohesive or adhesive and as it is an in vitro study, clinical evaluation is necessary to determinethe efficacy of these antioxidants.

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