ORIGINAL RESEARCH

Evaluation of the Effect of Different Antioxidants on Resin Bond Strength to Enamel after Bleaching: An *In Vitro* Study

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Abstract

Aim: To compare and evaluate the efficacy of 10% sodium ascorbate (SA) solution, 10% alpha-lipoic acid (ALA) solution, 10% propolis solutions (PLS), and 10% pomegranate peel extract (PPE) solution on the shear bond strength (SBS) between composite resin and bleached enamel after 60 minutes.

Materials and methods: Ninety extracted teeth were divided into 6 subgroups ($n = 15$) based on the antioxidant applied: Group I: Bleaching + application of 10% SA solution for 60 minutes. Group II: Bleaching + application of 10% ALA solution for 60 minutes. Group II: Bleaching + application of 10% PLS for 60 minutes. Group IV: Bleaching + application of 10% PPE solution for 60 min. Group V: Positive control (PC) bleaching without antioxidant application. Group VI: Negative control (NC) specimen neither subjected to bleaching nor application of an antioxidant solution. About 35% hydrogen peroxide (HP) was used for bleaching the labial surfaces in the groups considered. Labial surfaces were then bonded with composite using a customized cylindrical mold followed by storage in distilled water for 24 hours and shear bond strength was tested using universal testing machine.

Results: The mean shear bond strength values for groups I, II, III, IV, V, and VI are 5.04, 2.29, 5.02, 4.07, 1.42, and 9.08 respectively. The peak shear bond strength value is obtained for the negative control group; whereas the lowest mean SBS value is obtained for the positive control group.

Conclusion: All herbal extracts used in the present study have demonstrated a reversal of reduced SBS between resin composite and bleached enamel surface.

Clinical significance: The reduction in bond strength of adhesive restorations was conventionally overcome by delaying the bonding procedure for a specified period resulting in prolonged treatment duration and multiple appointments. Sodium ascorbate is most commonly used but it is postulated to form a porous scaffold capable of harboring bacteria. Herbal antioxidants are gaining popularity in this regard as they are naturally derived, effective and affordable phytochemicals in addition to being viable alternatives against drug-resistant strains of microorganisms.

Keywords: α-Lipoic acid, Herbal antioxidants, Pomegranate peel extract, Propolis, Shear bond strength, Sodium ascorbate.

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Introduction

One of the most common clinical issues related to esthetics is the pigmentation of dental structures. Changing times have resulted in changing trends and priorities among patients. Discoloration of teeth (extrinsic or intrinsic) can be managed by microabrasion, bleaching, composite resin restorations, and porcelain laminates or veneers utilized individually or in combination depending on the case. With an increasing understanding of esthetic dentistry, bleaching has become a very common procedure as it is conservative, economical, and delivers immediate results. Vital bleaching is a prevalent treatment option for discolored teeth and can be accomplished by using carbamide or hydrogen peroxide (HP) bleaching agents at different concentrations. The mechanism of bleaching agents is based on oxidation-reduction reactions. When applied on the tooth surface, hydrogen peroxide undergoes ionic dissociation and gives rise to the formation of free radicals such as hydroxyl radical, perhydroxyl, nascent oxygen, and superoxide anions, which are the most potent free radicals. These are extremely reactive and therefore react with the electron-rich regions of pigment within the tooth leading to the dissociation of the larger pigmented molecules into smaller and less pigmented molecules. The chemical process converts organic material into carbon dioxide and water. This reaction releases oxygen, which is a highly reactive free radical. This reaction creates a bleaching effect by penetrating the porosities of the enamel and converting the high-molecular-weight organic molecules in enamel prisms into low-molecular-weight compounds.[1–5]

A thorough knowledge of the outcome of the bleaching treatment and their impact on further dental procedures, especially in terms of adhesive restorations is indispensable. Several untoward effects are noted after bleaching such as increased tooth

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sensitivity, gingival irritation, posttreatment loss of adhesion, etc. Consequently, any subsequent restorative procedure has to be delayed resulting in prolonged treatment time.[1–3]

The reduction in bond strength of adhesive restorations to teeth surfaces is attributed to the presence of residual peroxide and oxygen layer which alters the resin polymerization. The broad approach to overcome this altered bond strength was to delay the bonding procedure by 24 hours to 3 weeks.[1–4] Antioxidants are agents that prevent oxidation by scavenging oxygen-free radicals from tooth surfaces thereby eliminating the untoward effect of a bleaching agent on the bond strength. Antioxidants obtained from natural as well as synthetic compounds have demonstrated adequate reversal of reduced bond strengths between composite resin and bleached enamel surfaces. Multiple active compounds like oligomeric-proanthocyanidins, flavonoids, phenolic compounds, etc. obtained from natural extracts act alone or in synergy resulting in the said beneficial effects. Consequently, unlike the conventional protocol of delaying the procedure, the use of an antioxidant allows further treatment to be carried out in the same appointment.[1–6] Therefore, with the aim of immediate reinstatement of esthetics, this study was conducted to compare and evaluate the efficacy of 10% sodium ascorbate (SA) solution, 10% alpha-lipoic acid (ALA) solution, 10% propolis solutions (PLS) and 10% pomegranate peel extract (PPE) solution on the shear bond strength (SBS) between composite resin and bleached enamel after 60 minutes.

Materials and Methods

This study was conducted over 3 months from June 2022 to August 2022 in the Department of Conservative Dentistry and Endodontics, Yenepoya Dental College, Yenepoya (Deemed to be University), Mangaluru, Karnataka, India. A total of 90 freshly extracted, permanent human anterior teeth were obtained for the study from the Department of Oral and Maxillofacial Surgery. The extracted teeth were rinsed under running distilled water and cleaned with an ultrasonic scaler to eliminate any stain, calculus, and remnants of periodontal tissue. All the samples were kept in 0.1% thymol solution for five days at 4°C followed by saline solution until use. The following criteria were followed for the selection of teeth. Previously untreated, caries-free permanent human anterior teeth, with intact enamel surfaces, extracted only for periodontal reasons were chosen for the study. Any teeth having carious lesions, restorations, history of endodontic or prosthodontic treatment, anomalies, or presence of cracks or fracture lines were excluded from the study.

Preparation of Solution

- Sodium ascorbate 10% (SA) – 10 grams of SA (Charco Chemicals, Hoshiarpur) dissolved in 100 mL of distilled water to make a 10% SA solution.
- Alpha lipoic acid 10% (ALA) – 10 grams of ALA (Onelife Nutriscience Pvt. Ltd, Mumbai) was dissolved in 100 mL ethanol to make a 10% ALA solution.
- Propolis 10% (PLS) – 10 grams of PLS powder (Hi-Tech Natural Products Ltd, Saharanpur, India) was dissolved in 100 mL of ethanol to make a 10% PLS solution.
- Pomegranate peel 10% (PPE) – 10 grams of PPE (Glamza Essential, Bhavnagar) was dissolved in 100 mL of distilled water to make a 10% solution of PPE.

Specimen Preparation

Within seven days of extraction, the teeth were embedded in acrylic resin with the labial surfaces facing upwards and roots completely embedded in resin. The labial surfaces were then sequentially polished using 400-, 600- and 1200-grit aluminium oxide abrasive papers for 30 seconds each under running water. This was followed by thorough ultrasonic cleaning of any leftover residues using distilled water.

The specimen thus prepared were randomly distributed into six groups based on experimental protocol and exposure to the antioxidant agent as follows:

- Group I: Bleaching (n = 15) followed by application of 10% SA solution for 60 minutes.
- Group II: Bleaching (n = 15) followed by application of 10% ALA solution for 60 minutes.
- Group III: Bleaching (n = 15) followed by application of 10% PLS for 60 minutes.
- Group IV: Bleaching (n = 15) followed by application of 10% PPE solution for 60 minutes.
- Group V: Positive control (PC) (n = 15) bleaching procedure performed without subsequent antioxidant application.
- Group VI: Negative control (NC) (n = 15) specimen were neither subjected to bleaching nor application of any antioxidant solution.

Labial surfaces of each specimen belonging to groups I to V (n = 75) were subjected to bleaching using 35% hydrogen peroxide for 8 minutes according to the manufacturer's instructions. The bleaching gel was then completely rinsed off using distilled water. The respective antioxidant solution was consecutively applied for 60 minutes and the solution was refreshed every 10 minutes followed by the bonding procedure.

Bonding Procedure

Labial surfaces of specimens (n = 90) were etched using 37% phosphoric acid and rinsed after 15 seconds and dried using absorbent paper. The bonding agent was applied using an applicator tip in two successive layers with 5 seconds of air spray to evaporate the excess solvent. A light-emitting diode (LED) light (Bluephase, Ivoclar Vivadent AG) with an intensity of 1200 mW/cm^2 was used to polymerize the material for 20 seconds. The tip was held in close contact at right angles to ensure uniform curing standardized at a distance of 1 mm between the material and tip surface, while the standardization of light intensity was ensured using a radiometer. Resin composite build-up was performed in the form of cylinders using a stainlesssteel mold of dimensions 3 mm diameter and 5 mm height followed by polymerization for 40 seconds using the same curing light. All samples were stored in distilled water at 37°C for 24 hours followed by SBS testing.

Shear Bond Strength Testing

All specimens were fixed on the jig of a universal testing machine and the knife-edged shearing rod was utilized at a crosshead speed of 1 mm/minute. The load required to dislodge the composite cylinders on each specimen was logged in MPa and the data thus acquired was subjected to statistical analysis.

Statistical Analysis

Statistical analysis was performed using IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp. Results on continuous

Table 1: Comparison of shear bond strength (Mpa) between the groups

	N	*Minimum*	*Maximum*	*Mean*	*SD*	*p-value*
Group I (SA)	15	4.73	5.26	5.04	0.15	0.001*
Group II (ALA)	15	1.54	2.71	2.29	0.37	
Group III (PLS)	15	4.32	5.18	5.02	0.23	
Group IV (PPE)	15	3.81	4.24	4.07	0.14	
Group V (PC)	15	0.96	1.78	1.42	0.25	
Group VI (NC)	15	8.06	9.75	9.08	0.62	

Table 2: *Post-hoc* comparison of shear bond strength (MPa) between two groups

Group	*Group*	*Mean difference*	*Std. error*	*p-value*
Group I	Group II	2.74	0.12	0.001*
	Group III	0.02	0.12	0.838
	Group IV	0.96	0.12	0.001*
	Group V	3.62	0.12	0.001*
	Group VI	–4.04	0.12	0.001*
Group II	Group III	–2.72	0.12	0.001*
	Group IV	–1.78	0.12	0.001*
	Group V	0.86	0.12	0.001*
	Group VI	–6.79	0.12	0.001*
Group III	Group IV	0.94	0.12	0.001*
	Group V	3.59	0.12	0.001*
	Group VI	–4.06	0.12	0.001*
Group IV	Group V	2.65	0.12	0.001*
	Group VI	–5.00	0.12	0.001*
Group V	Group VI	–7.65	0.12	0.001*

measurement are presented as Mean and SD. Inferential statistics like the Kruskal-Wallis test were used to compare the groups. *Post-hoc* analysis was performed using the Mann-Whitney *U* test. A *p*-value less than 0.05 was considered statistically significant. Bonferroni adjustment was done for the *post-hoc* test resulting in a *p*-value of 0.003 (0.05/15).

Results

The mean shear bond strength values for groups I, II, III, IV, V, and VI are 5.04, 2.29, 5.02, 4.07, 1.42, and 9.08 respectively. The highest mean shear bond strength value was obtained for group VI (NC) group, followed by groups I, II, IV, and II respectively, whereas the lowest mean shear bond strength value was obtained for group V (PC) as demonstrated in Table 1. A statistically significant difference was observed between all the groups except for the difference between Group I and Group II as demonstrated in Table 2.

Discussion

Bleaching of teeth can be performed extracoronally on vital teeth called vital tooth bleaching or intracoronally in rootfilled teeth, called nonvital tooth bleaching. It is one of the most common, effective, and affordable esthetic procedures which finds marked patient acceptance and satisfaction. Bleaching is performed these days either alone or in combination with other esthetic procedures and has thereby widened the horizons for the practice of esthetic dentistry. It is noteworthy that satisfactory outcomes derived from bleaching procedures, motivate the patients to opt for additional esthetic procedures like veneers, diastema closures, laminates, etc.[2]

As the bleaching agent essentially changes the structure and composition of the pigments on the teeth and its efficacy is concentration and time-dependent; it alters the morphology and chemical structure of the enamel. This is reflected in the form of reduced shear bond strength between the adhesive restorative material and the tooth surface as indicated in several *in-vitro* studies. Rotstein et al. suggested altered levels of calcium and phosphorus in the hydroxyapatite crystals.[5] According to research, reduction in bond strength can be due to the variations in protein and mineral content of the superficial layers of enamel. Others attributed the compromised bond strength to less numerous, shorter, and less defined resin tags in bleached enamel, interference of residual peroxide forming gas bubbles, and inhibition of polymerization.[1,7]

Several techniques have been recommended to resolve the clinical complications related to compromised bond strength; like treatment of the bleached surface with alcohol before restoration, elimination of the superficial layer of enamel, and use of adhesives comprising organic solvents. However, the broad approach is to postpone any adhesive procedure for a period varying between 4 hours to 4 weeks after bleaching, because the reduction in bond strength is temporary.[8,9]

Various authors have suggested that the application of an antioxidant can also successfully compensate for the decreased bond strength and that the duration of application of an antioxidant may vary.[10,11] According to Lai et al. antioxidant has to be applied for no less than one third of the bleaching time for it to completely alter its effects corresponding to approximately 3 hours.[12] Research data indicates that increased duration of antioxidant application results in successful reversal of altered bond strength. In situations where it is not feasible to wait for a longer period, antioxidant application for 60 minutes can prove to be an effective alternative.[10,13] Further, the research data suggests that SA can be used in both gel and solution forms and Kimyai and Valizadeh.[14] demonstrated similar effect with the non-significant difference between them. Therefore, a 10% SA solution was chosen for 60-minute application period in the present study.

In the present study, treatment of the bleached surfaces with 10% SA solution before bonding appeared to restore the decreased SBS of composite resin to the enamel. The results are by the previous studies conducted by Bansal et al. and Arumugam et al., where similar effects were observed by antioxidant treatment.[15,16] The conceivable explanation for this may be the antioxidant ability of SA which enables neutralization and reverses the oxidizing effects of bleaching agents. Furthermore, SA allows adhesive polymerization to progress without premature termination by reinstating the altered redox potential of the oxidized substrate. The enamel is exposed to air and may include oxygen along with water and organic molecules. The oxygen adjacent to the unbleached enamel might also inhibit polymerization at the resin/enamel interface which is reduced by a potent antioxidant like SA.[2,4,15] Thus, the higher SBS in specimens treated with SA than in unbleached specimens may be attributed to its neutralizing ability.

The literature describes the use of various other antioxidants like α-tocopherol, grape seed extract, lycopene, oligomeric-proanthocyanidin, aloe vera, etc. but these studies are scarce and to the best of our understanding, none have equated the efficacy of PPE, PLS, ALA and SA. Therefore, the present study aimed to assess the effects and efficiency of 10% SA solution, 10% ALA solution, 10% PLS solutions and 10% PPE solution on the SBS between composite resin and bleached enamel after 60 minutes.

Testing of bond strength in smaller specimens can be performed using TBS and SBS tests. According to research both tensile and shear bond tests are equally effective in assessing bond efficiency. The SBS tests are more commonly used due to their validity and relatively straightforward method. μTBS requires slicing which results in structural defects causing premature failure often before testing of the specimen. Therefore, SBS testing was utilized in the present study as it results in uniform stress distribution, has a simple process of specimen preparation resulting in the conservation of tooth structure, identifies irregular bonding interface, has a universal fixture, and allows observation under a microscope to identify modes of failure after a fracture. All of which result in feasible testing and reliable results.[17,18] In this study the mean SBS values of different antioxidants in decreasing order were – SA > PLS > PPE > ALA.

Alpha-lipoic acid is soluble in both lipid and aqueous medium thus making it a unique antioxidant; but, it is highly soluble in organic solvents and only partially soluble in water which is why in the present study it was dissolved in ethanol. It is capable of scavenging various types of free radicals owing to its sulfur structure. It is referred to as the universal antioxidant as it participates in various activities of the body. Antioxidants reinstate the altered redox potential of the oxidized substrates and allow adhesive polymerization to progress without premature termination leading to the restoration of the compromised bonding.[18] According to the outcomes of this study, all antioxidant agents used were able to restore the compromised SBS after bleaching and this effect was statistically significant.

Pomegranates have a prevailing medical history and hold extraordinary medicinal properties. The pomegranate peels constitute 50% of total fruit weight, they are a prominent source of bioactive compounds and minerals. Pomegranate peels are a probable source of phenolics, flavonoids, and proanthocyanidins. The phytochemical ethanolic extract has a high inhibitory effect against both *gram-negative* and *gram-positive bacteria*.[19] In a study, Sharafeddin and Farshad[20] used PPE at two different concentrations between 5 and 10% to compensate for the negative impacts of free radical ions on the bonding agent and concurred that the two concentrations were equally efficacious on SBS of composite to bleached enamel. The results of our study also demonstrated improved shear bond strength thereby validating the effect of PPE.

Propolis is a resinous extract from bee glue and has proven antioxidant properties owing to the presence of flavonoids. According to Goncalves et al.,[21] a 2% PLS extract demonstrated antioxidant activity even at low concentrations in addition to antimicrobial activity. An ethanolic extract of PLS was used at the rate of one mL/min for 10 minutes in their study which was similar to the application of SA and showed improved tensile bond strength of the composite. An additional benefit was noted in the form of deeper resin tag penetration and increased hybrid layer thickness. However, an aqueous extract of PLS is known to exhibit better antioxidant properties and there is a dearth of literature regarding the same; therefore, in the present study, a 10% aqueous extract of PLS was utilized and it has shown a significant reversal of reduced SBS but the difference was statistically non-significant when evaluated with other groups.[22,23]

Limitations and Future Perspectives

This study was conducted in a controlled lab setting following which additional long-term *in vivo* and *in vitro* studies are recommended to evaluate the effect of the antioxidant application. The impact of different bonding systems was not taken into account and therefore, further research can be directed towards comparative evaluation among recent bonding systems and newer restorative materials. The comparisons were restricted to the enamel and dentin was not included hence further research can evaluate SBS of resin-dentin interface and antioxidant application as it may be relevant in cases undergoing non-vital bleaching.

Conclusion

All herbal extracts used in the present study are biocompatible reducing agents that are used in edible foodstuffs and have not been reported to cause any harmful effects on the enamel surface. Furthermore, they have demonstrated the reversal of reduced shear bond strength between resin composite and bleached enamel surface. However, the present study was carried out in controlled laboratory settings following which clinical extrapolations of results must be made discretely.

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