

In-Vitro Evaluation of the Effect of Sage on Shear Bond Strength of Composite Resin to Bleached Enamel

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Abstract

Background and Aim: There is a reduction in bond strength of composite resin to bleached enamel. The aim of the present study was to evaluate the effect of sage (*Salvia officinalis*) on the shear bond strength of composite resin to bleached enamel.

Materials and Methods: In this in vitro study, 60 labial surfaces of maxillary incisors were randomly divided into 4 groups as follows: G1: bleaching; G2: bleaching and application of sodium ascorbate-containing solution; G3: bleaching and application of *Salvia officinalis*-containing solution; G4: control (no bleaching). A composite resin (Z100; 3M ESPE, Dental products, St Paul, MN, USA) cylinder was bonded on each specimen after acid etching and application of a fifth generation bonding agent (Single Bond; 3M ESPE, Dental products, St Paul, MN, USA). After thermocycling, the shear bond strengths of the samples were measured in MPa. Data were analyzed using one-way ANOVA and Tukey's HSD test ($\alpha=0.05$).

Results: Minimum and maximum mean values of shear bond strengths were observed in G1 (12.31 ± 2.44) and G3 (25.04 ± 3.52), respectively. No statistically significant differences were found in bond strength between the bleached and non-bleached groups after the antioxidant treatment ($p>0.05$).

Conclusion: There is a considerable decrease in bond strength of composite resin to enamel immediately after bleaching. Application of sodium ascorbate and *Salvia officinalis* solution can increase the bond strength of composite resin to bleached enamel.

Key Words: Antioxidants, Composite resin, Sodium ascorbate, *Salvia officinalis*, Tooth bleaching, Bond strength

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Introduction

At present, due to an increasing demand for tooth bleaching procedures, clinicians should be well aware of the effect of this technique on other dental treatments, especially on bonded restorations [1]. In the majority of bleaching procedures, derivatives of hydrogen peroxide are used at different concentrations. It seems that the mechanism of bleaching is based on an oxidation reaction in organic pigments [2].

Usually after the tooth bleaching procedures, patients ask for other esthetic treatments, namely re-

placement of old composite restorations and exchange of amalgam fillings with tooth-colored restorations. After the bleaching procedure, residual oxygen and remnants of hydrogen peroxide are still present in tooth structures and prevent the polymerization of the adhesive resin and formation of resin tags in the etched enamel. Clearly, this issue influences the bond strength and compromises the long-term service of restorations [3,4].

However, since the decrease in bond strength is transient, some dental practitioners delay the bonding procedures for 1-4 weeks [5,6]. Since bleaching

can compromise the bonding of composite resin restorations, it is necessary to adopt measures to achieve adequate bond strength for such restorations. One suggested technique is the use of anti-oxidative agents and several studies have evaluated this technique [7, 8].

Ascorbic acid and its salts (sodium ascorbate) are the most commonly used antioxidants for the bleached teeth. These agents can restore the bond strength of bleached teeth to that of non-bleached teeth. A large number of studies have evaluated the effect of different forms and concentrations of sodium ascorbate and have shown promising results. [4,5,9].

Regardless of the wide-spread application of sodium ascorbate as an antioxidant, Oskuee et al. showed that the cumulative effect of sodium ascorbate and bleached material may lead to maximum retention of pathogenic microorganisms in enamel surfaces [10].

On the other hand, the increasing tendency to use herbal products in medical fields encourages researchers to evaluate the clinical efficacy of suitable plant-derived alternatives to commonly used chemical agents.

Salvia officinalis is a plant-derived antioxidant that has been the focus of attention in Greek and Roman traditional medicine. Several therapeutic effects have been described for this medicinal plant, namely treatment of pharyngitis and Alzheimer's disease and relieving mental tension [11,12] Also, it possesses antibacterial and anti-inflammatory properties [13,14].

Studies have confirmed the anticarcinogenic and antidiabetic effects of this antioxidative agent [15,16].

Salvia officinalis extract is used as a mouthwash in order to control dental plaque and gingivitis in patients [17] In addition, the antimicrobial activity of sage against oral pathogens decreases oral malodor [18] It also has antibacterial activity against *Streptococcus mutans* [19] Although the antioxidative effect of this extract has been shown in medicine, only a few studies are available on its efficacy in dentistry. This study was carried out to evaluate the effect of sage (*Salvia officinalis*) on shear bond strength of composite resin to bleached enamel.

Materials and Methods

In this in-vitro study, 60 maxillary anterior teeth extracted 3 months before the study were selected. The teeth had no cracks, carious lesions or coronal restorations. After removing the tissue remnants, the teeth were rinsed under running water and immersed in 0.5% chloramine T solution. Then the teeth were immersed in distilled water (Shahid Ghazi Co., Tabriz, Iran) at room temperature for 24 hours before the experiment. The roots were cut at 2 mm apical to the CEJ using M016-878 diamond bur (SS White Inc, Lack Wood, USA) and high-speed handpiece with air and water spray. The pulp of the coronal segment was removed. The samples were placed in molds measuring 35×25×10 mm, containing translucent acrylic resin (Marlic Med Co., Tehran, Iran), with the labial surfaces facing outward. The samples were placed in cold water in order to control the heat generated during the polymerization of the acrylic resin. The enamel labial surfaces with 4×4 mm dimensions were polished with wet 400- and 600-grit silicone papers to achieve a flat homogeneous labial surface in all specimens.

Subsequently, the specimens were randomly divided into 4 equal groups:

Group 1: The enamel surfaces were immediately bonded after bleaching.

Group 2: The bleached enamel surfaces were immediately treated with 10% sodium ascorbate solution and bonded.

Group 3: The bleached enamel surfaces were immediately treated with 10% sage (*Salvia officinalis*) solution and bonded.

Group 4: The specimens were not bleached and bonded.

The bleaching procedure consisted of the application of 16% carbamide peroxide gel (Opalescence, Ultradent Inc., USA) for 8 hours per day with a microbrush, based on the manufacturer's instructions in groups 1 to 3. The samples were immersed in artificial saliva, containing 1 g of carboxymethyl sodium cellulose, 4.3 g of xylitol, 0.1 g of potassium chlorite, 5 mg of calcium chloride, 40 mg of potassium phosphate, 1 mg of potassium thiocyanate, and 100 g of distilled water (pH=7) in a manner so that the bleached enamel surface had no contact with saliva. At the end of each bleaching session, the samples were thoroughly rinsed with air/water spray for 30 seconds and air-dried. Then

the samples were immersed in 250 mL of artificial saliva for the rest of the 24-hour period, i.e. for 16 hours. The bleaching procedure was continued for 14 days.

The maceration technique was used for the anti-oxidant extraction. At first, the previously dried aerial branches were weighed using a digital weighing machine (Lib POR AEU-210) to select 50 g of the plant. The dried branches were powdered and placed in an Erlenmeyer flask. Each sample was completely immersed in 1500 mL of a mixture of water and ethanol (50% of 99% ethanol and 50% of water). Aluminum plates were placed on Erlenmeyer flasks and transferred to a shaker (Heidolph Unimax 2010) for 48 hours at 90 rpm. After the solvent and the plant were homogenized the solution was filtered through the filter papers (Whatman, 0.5 mm, USA).

The solutions were then transferred to a rotary evaporator (Heidolph WD 2000) to separate the solvent from the extract 20; 10% solution was prepared from the purified extract (>99%) for use. In groups 2 and 3, after the bleaching process the samples were immediately placed in 10 mL of 10% sodium ascorbate and 10% sage solution for 10 minutes, respectively. Then, the samples were rinsed with distilled water and dried.

In group 4, the samples were not subjected to bleaching or antioxidant application.

The enamel surfaces of all samples were etched with 35% phosphoric acid gel (Scotch Bond Etchant, 3M ESPE, Dental Products, St. Paul, MN, USA). Then an etch-and-rinse bonding agent (Single Bond, 3M ESPE, Dental Products, St. Paul, MN, USA) was applied, dried with gentle air current for 5 seconds and light-cured for 20 seconds using a light-curing unit (Hilux LED 550, Benlioglu, Dental, Ankara, Turkey).

Transparent plastic molds, with an inner diameter of 5 mm and a height of 4 mm, were placed on prepared enamel surfaces. Light-curing composite resin (Z100, 3M ESPE, Dental Products, St. Paul, MN, USA) was placed inside the molds in 1-mm increments and each layer was light-cured from two sides of each mold for 20 seconds at an output power of 500 mW/mm². For evaluation of shear bond strength universal testing machine (Zwick, Berlin, Germany) was used. Load was applied to

tooth-composite interface parallel to the long axis of the tooth crown at a crosshead speed of 0.5 mm/min until the samples fractured. After calculating the bond strength values, data were analyzed by SPSS version 15 software using one-way ANOVA and Tukey's HSD test. Statistical significance was set at $P < 0.05$.

After the shear bond strength test, the fractured surfaces of samples in each group were evaluated under a stereomicroscope (Nikon Eclipse E600, Tokyo, Japan) at $\times 20$ magnification. The mode of fracture was classified as follows:

1. Fracture within the enamel or the composite resin (cohesive)
2. Fracture at enamel-composite resin interface (adhesive)
3. Mixed fracture

For morphological evaluation of fractured surfaces, one sample from each group was placed on the holding device of the equipment and sputter-coated with gold-palladium by a sputter coater (Model SC7620) in order to create a layer capable of conducting electricity. Then, the surfaces were evaluated under a SEM (Nikon Eclipse E600, Tokyo, Japan) at different magnifications.

Results

Groups 1 and 4 exhibited the lowest and highest bond strength values, respectively. Table 1 presents means, standard deviations and maximum and minimum values in each group.

One-way ANOVA revealed statistically significant differences between the groups ($p < 0.001$). Pair-wise comparison of groups showed that differences in the shear bond strength values between the groups were not significant except for the differences between groups 1 and the other groups ($p > 0.05$) (Table 2).

Table 3 presents the frequency distribution of fracture modes in each group determined under stereomicroscopic.

Morphologic evaluation of the surface under a scanning electron microscope at different magnifications showed that in group 1 the adhesive resin on the enamel surface seemed porous. However, in the other experimental groups it was more homogeneous and without voids (Figures 1 and 2)

Table 1. The mean, SD, minimum and maximum bond strength values (MPa) in the groups studied

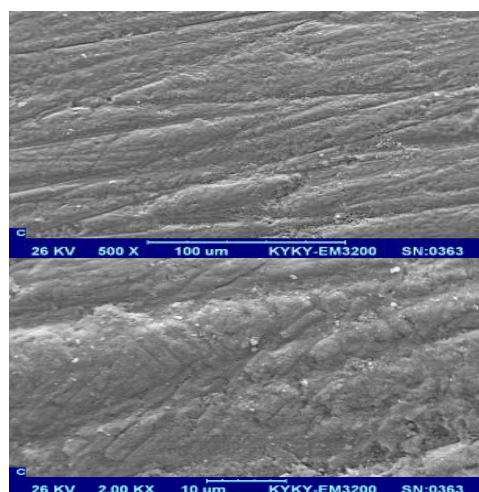
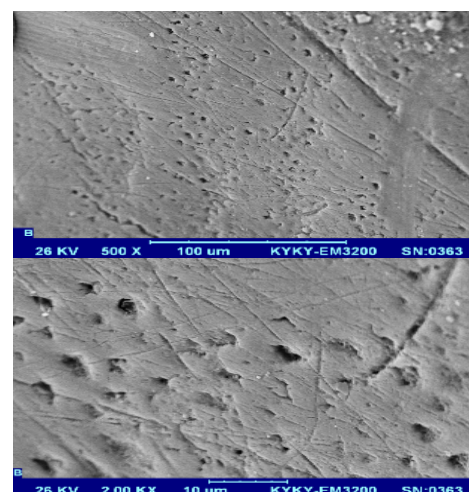
Group	N	Mean	SD	Maximum	Minimum
1	15	12/31	2/44	16/23	8/72
2	15	23/98	3/52	28/93	18/19
3	15	25/04	4/20	31/35	18/71
4	15	21/97	1/62	25/53	19/05
Total	60	20/82	5/91	31/35	8/72

Table 2. Paired comparison of bond strength values between groups

Group	Group	Mean difference	SD	P value
1	2	11/6700	1/1367	*0/000
	3	12/7380	1/1367	*0/000
	4	9/6586	1/1367	*0/000
2	3	2/0113	1/1367	0/299
	4	1/0680	1/1367	0/784
3	4	3/0793	1/1367	0/053

Table 3. Paired comparison of bond strength values between groups

Group	Adhesive	Cohesive	Mixed
1	13 (86.7%)	0	2 (13.3%)
2	9 (60%)	0	6 (40%)
3	9 (60%)	0	6 (40%)
4	10 (67%)	0	5 (33%)

**Figure 1.** Evaluation of surface morphology shows a uniform, void-free adhesive layer on the enamel surface in group 3 (500X and 2000X magnifications)**Figure 2.** Evaluation of surface morphology shows a porous adhesive layer with voids in group 1 (500X and 2000X magnifications)

Discussion

Use of antioxidative agents is a routine method for compensating the reduced bond strength of composite resin restorations to tooth structure. Studies have reported the antioxidative effect of sage (*Salvia officinalis*) [21,22]. Therefore, the present study was undertaken to evaluate the effect of sage on the shear bond strength of composite resin to bleached enamel.

Based on the results of the present study, the lowest bond strength values were observed in group 1, compared to the other groups. Some studies have shown that enamel structure undergoes some physical changes due to the effect of bleaching agents and the decrease in bond strength is attributed to an increase in enamel surface porosity due to over-etching and loss of the crystalline structure of the enamel [5]. One study showed that the decrease in bond strength after application of bleaching agents on enamel is due to a decrease in the calcium content of enamel, a decrease in enamel surface hardness and destruction of enamel structure [23].

Rotstein et al. reported a decrease in the strength and solubility of enamel, dentin and cementum after bleaching procedures [24]. Changes in the mineral and organic content of teeth are attributed to an increase in solubility. Some other studies have attributed an increase in enamel porosity to the retention of oxygen in the form of free radicals. In general, the results of the present study are consistent with those of Kaya et al, Bulut et al, and Khoroshi et al [3,4,6]. It has been suggested that storage of bleached specimens in the saliva or a humid environment can gradually release the residual oxygen in the form of liquid or gas until it is completely eliminated.

Another finding of the present study was that the use of sodium ascorbate and sage solutions in groups 2 and 3 significantly increased the bond strength but with no significant difference from the control group (no bleaching). It means that application of the mentioned two materials on the bleached tooth surfaces neutralizes the free radicals trapped in tooth structure and increases the bond strength of composite resin to enamel.

Many studies have confirmed the effects of sodium ascorbate as an antioxidant agent. Thus, it was selected as a standard antioxidative agent in this study [4-8]. The increased bond strength in group 3

may be attribute to the antioxidative property of sage extract.

The mechanism of action of sage extract has yet to be fully understood; but polyphenols such as ursolic acid, caffeic acid, hispidulin, rosmanol, apigenin, carnosic acid and carnosol have been recognized as the active ingredients of this material preventing the formation of reactive oxygen species [25,26]. Antioxidants neutralize free radicals by donating one electron to these agents. However, neutralized antioxidants are not converted into free radicals by giving away one of their electrons because they are stable in any form [27]. Studies have confirmed beneficial biologic effects of this material and it appears to have no side effect on the orodental system [17-19].

Stereomicroscopic evaluation of fractured surfaces in group 1 showed adhesive fracture mode in 86% of the cases. However, in other groups about 67% of specimens had mixed fracture mode. Leloup reported a strong relationship between the mean bond strength and fracture mode and concluded that there was an increase in the incidence of mixed and cohesive fracture modes with an increase in bond strength [28]. Similarly, in our study the results of stereomicroscopic evaluations confirmed the results of bond strength. Morphologic evaluation of the surface by SEM showed numerous voids in the area with low bond strength in group 1 specimens. Titley et al. reported that the resin bleached enamel interface is different under SEM compared to the resin non-bleached enamel interface. In bleached enamel a large area on the enamel surface was devoid of resin. In addition, in areas where resin tags were present, they had a fractured pattern and shallow penetration into the enamel compared to the non-bleached enamel [28]. Furthermore, the resin-bleached enamel interface exhibited a porous and granular structure. This bubble-like appearance in our study was due to oxygen remaining in the enamel structure. On the other hand, use of antioxidants eliminates the residual oxygen; which is responsible for the homogeneous resin enamel interface without any bubbles in other experimental groups.

A study reported that sodium ascorbate gel at concentrations higher than 10% can restore the decreased bond strength of bleached teeth [29]. Since the minimum duration of application of antioxidants to the bleached enamel had not been men-

tioned in any study, these agents were applied for 10 minutes in our study according to Turkan et al, [29] and Kaya et al [6]

In our study, 16% carbamide peroxide was used for external bleaching of teeth. In some studies 16%, 20% and 22% concentrations of carbamide peroxide have been used [5-7] and some others have suggested the use of 35% hydrogen peroxide [9]. Since the solution containing sage was colorless, no discoloration was caused in the enamel.

The present study was a preliminary study in this respect and we applied sage extract in the form of solution using the immersion technique. However, the hydrogel form is preferred over the solution due to easier control and handling. The hydrogel form is more suitable for clinical application and its efficacy should be compared with that of the solution form. Since antioxidants have short shelf lives and become inactive after some time, further studies are suggested on storage techniques of these materials.

Conclusion

Bleaching significantly decreases the shear bond strength of composite resin to enamel. The bond strength of composite resin to bleached enamel significantly increases with the use of sage and sodium ascorbate solutions.

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