ORIGINAL ARTICLE

Journal of Research in Dental and Maxillofacial Sciences

DOI: 10.52547/jrdms.7.2.62

Effect of Pomegranate Peel and Green Tea Extract as Antioxidants on Shear Bond Strength of a Microhybrid Composite to Bleached Enamel

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Article History
Received: 22 August 2021
Accepted: 2 January 2022

Abstract

Background and Aim: This study evaluated the effect of pomegranate peel and green tea extracts on shear bond strength (SBS) of a microhybrid composite to bleached enamel.

Materials and Methods: In this in vitro, experimental study, 44 maxillary premolars were divided into 4 groups of control A (composite cylinders bonded to teeth without bleaching), group B (composite bonding immediately after bleaching), group C (5 g of green tea extract was dissolved in 100 mL of distilled water, applied for 10 minutes, and rinsed for 30 seconds, followed by bleaching and composite bonding) and group D (5 g of pomegranate peel extract was dissolved in 100 mL of distilled water; the rest was similar to group C). In groups B, C and D, 35% hydrogen peroxide was applied on the teeth for 20 minutes and rinsed. This process was repeated two more times. The teeth were etched with 35% phosphoric acid for 15 seconds and rinsed for 15 seconds. A microhybrid composite was applied in Tygon tubes, bonded to the teeth with single Bond, and light-cured. Mode of failure was also determined. SBS was measured by a universal testing machine and data were analyzed by one-way ANOVA and Tukey's test.

Results: A statistically significant difference was found in the mean SBS of the groups ($P<0.001$). The mean SBS of group B (not treated with antioxidant after bleaching) was significantly lower than that in other groups ($P<0.05$).

Conclusion: Application of 5% pomegranate peel and green tea extracts increase the SBS of composite to bleached enamel.

Key Words: Shear strength, Antioxidants, Dental Enamel, Tooth bleaching

Cite this article as: Ghorbani F, Pourhaghani S, Heshmat H, Jalalian S, Kharazifard MJ. Effect of Pomegranate Peel and Green Tea Extract as Antioxidants on Shear BondStrength of a Microhybrid Composite to Bleached Enamel.
J Res Dent Maxillofac Sci. 2022; 7(2):62-69.

Introduction

Esthetic appearance of anterior teeth is an important topic in modern dentistry [1]. Vital tooth bleaching is a safe and well-accepted procedure for treatment of superficial and intrinsic stains [2]. The bleaching agents for in-office use contain high concentrations of carbamide peroxide (35% to 37%) or

hydrogen peroxide (30% to 35%); while at-home bleaching agents include low concentrations of both peroxides that are applied in a custom tray and used under the supervision of a dentist [3]. Hydrogen peroxide has an adverse effect on bond strength of composite to etched enamel when bonding agent is applied immediately after bleaching [4]. A widely accepted explanation for this adverse effect is diffusion of hydrogen peroxide into the organic matrix of enamel and dentin due to its low molecular weight, and release of free radicals, such as hydroxyl radicals, super oxide anions, singlet oxygen and hydroperoxyl that can react with unsaturated bonds, and break down the large organic molecules. These free radicals interfere with the formation of resin tags and adhesive polymerization [4]. Scanning electron microscopic examinations have shown defective shallow resin tags at the bonding interface in bleached, compared with non-bleached, enamel [5,6]. However, after a certain period of time, the residual oxygen gradually disappears, and resin can favorably bond to the bleached enamel [7,8]. The waiting period for bonding procedures after bleaching has been reported to vary from 24 hours to 4 weeks [2]. Bulut et al. [2] recommended at least 1 week interval between bleaching and bonding process.

A variety of methods have been proposed to neutralize the reduced bond strength of composite to bleached enamel. These methods include pre-treatment of the bleached enamel with alcohol before the bonding procedure, removal of superficial enamel, and using a natural solvent containing adhesives. Other methods include immersion of bleached enamel in distilled water, saline or artificial saliva (in vitro). Antioxidants such as 10% sodium ascorbate have been used to reverse the reduced bond strength to bleached enamel [1,3]. To eliminate clinical problems related to post-bleached compromised bond strength, some techniques have been suggested. Antioxidants are not converted to free radicals due to their stability in their present form. Subramonian et al. [8] compared the effects of 10% sodium ascorbate and grape seed extract and stated that antioxidants result in significantly greater bond strength than the bleached control group. Antioxidant treatment of bleached enamel before immediate bonding with composite resin appeared to restore the reduced tensile bond strength in their study. Also, Vidhya et al. [4] evaluated the effect of grape seed extract on bond strength of bleached enamel to composite resin, and reported that application of grape seed extract affected the bonding process of bleached enamel and significantly increased the bond strength. Pomegranate peel extract and green tea are non-toxic reducing agents that are used in food industry and are unlikely to have harmful biological effects on the enamel surface.

This study evaluated the effect of pomegranate peel and green tea extracts on shear bond strength (SBS) of a microhybrid composite to bleached enamel.

Materials and Methods

Preparation of specimens

For this in vitro experimental study, we collected 44 sound human maxillary premolars extracted for orthodontic reasons (ethical approval code: IR.IAU .DENTAL.REC. 1399.196). They had sound buccal enamel with no caries and no history of chemical exposure [2]. Right after extraction, all residual tissue tags were removed, and the teeth were cleaned with pumice, and rinsed under running tab water [3]. They were then stored in distilled water at room temperature [1]. The teeth were then mounted in acrylic resin blocks to the level of their cementoenamel junction such that their crown was exposed. The labial surface of the teeth was flattened with 600-grit silicon carbide abrasive paper and low-speed headpiece under constant water coolant [9].

Experimental groups

All samples were randomly categorized into 4 groups of 11 teeth:

Group A: No bleaching was performed, and the composite cylinders were bonded to the labial surface of the teeth (control group).

Group B: Composite cylinders were bonded to the labial surface of the teeth immediately after bleaching.

Group C: To prepare a solution of 5% green tea extract, first 5 g of dried green tea extract (CAMGREEN Co., P.E.G, Gorgan, Iran) was dissolved in 100 mL of distilled water and applied on the labial surface of the teeth for 10 minutes right after bleaching and was then rinsed off for 30 seconds. Composite cylinders were then bonded to the labial surface [3].

Group D: To prepare a solution of 5% pomegranate peel extract, first 5 g of dried pomegranate peel extract (Anar, Amin Pharmaceutical Co., Isfahan, Iran) was dissolved in 100 mL of distilled water and applied on the labial surface of the teeth for 10 minutes immediately after bleaching. It was then rinsed off for 30 seconds and the composite cylinders were bonded to the labial surface of the teeth [3].

Bleaching procedure:

In all three bleaching groups, a commercial 35% hydrogen peroxide gel (Whiteness, Co. FGM, Brazil) was applied on the enamel surface for 20 minutes in accordance with the manufacturer's instructions. Then, the gel was washed thoroughly with air/water spray and the process was repeated twice more [10].

Restoration process:

Preparation of the composite blocks was the same for all groups. For preparation of composite blocks, semi-transparent plastic tubes with a diameter of 4 mm and a length of 6 mm were used. Composite was applied in the tubes to obtain cylindrical specimens of the same size [11]. The restoration process was the same for all groups; the labial surface of the teeth was etched with 35% phosphoric acid for 15 seconds (Morvabon, Iran) according to the manufacturer's instructions, and then washed with water for 15 seconds. A fifth generation adhesive system (Single Bond; 3M ESPE, St. Paul, MN, USA) was applied in two successive layers, spread gently with air for 5 seconds, and light-cured with a curing unit (Bluephase, Ivoclar Vivadent, Lichtenstein) with a wavelength of 486 nm and light intensity of 1200 mW /cm^2 at 1 mm distance for 20 seconds [12] Then, microhybrid composite (A2 shade; Filtek Z 250; 3M ESPE, St. Paul, MN, USA) cylinders (5 mm diameter and 2 mm height) were placed on the bonding surface, and light-cured for 40 seconds at 1 mm distance from all directions. The curing unit was checked before use by a radiometer (Bluephase, Ivoclar Vivadent, Lichtenstein) [1]. Prior to the test, all samples were stored in distilled water for 24 hours. All teeth were placed in a universal testing machine, (Zwick Roell Pvt. Ltd, Berlin, Germany) to measure the SBS. The load was applied with a crosshead speed of 1 mm/minute to the composite-enamel interface with a knife-edged shearing rod [13]. The SBS was converted to megapascals (MPs) and reported.

A stereomicroscope (Olympus, Tokyo, Japan) was used for assessment of the failure mode at x40 magnification. It was categorized as adhesive (between the tooth and restorative material), cohesive (within the restorative material), or mixed. The data were analyzed by SPSS version 25 using one-way ANOVA and Tukey's test (alpha=0.05)

Results

The assumption of homogeneity of variances was met for the SBS data (P>0.05). Table 1 presents the SBS data in the four groups.

One-way ANOVA revealed a statistically significant difference in the mean SBS among the groups (P<0.001) (Table 2).

Table 1. Measures of central dispersion for SBS (MPa)

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval For Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Group A (Control)	11	22.0976	2.24668	0.67740	20.5883	23.6070	17.78	24.80
Group B	11	9.9300	1.32419	0.39926	9.0404	10.8196	7.36	11.82
Group C (Pomegranate)	11	22.1800	2.28642	0.68938	20.6440	23.7160	18.05	26.54
Group D (Green tea)	11	22.0109	2.88214	0.86900	20.0747	23.9472	19.05	27.87
Total	44	19.0546	5.75598	0.86775	17.3047	20.8046	7.36	27.87

Table 2. Results of one-way ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1221.289	3	407.096	80.076	0.000
Within Groups	203.355	40	5.084		
Total	1424.644	43			

Pairwise comparisons showed that the mean SBS in group B (not treated with antioxidant afterbleaching) was significantly lower than that in other groups ($P<0.05$). No other significant differences were noted ($P>0.05$). Table 3 presents pairwise comparisons of SBS (MPa). Table 4 presents the modes of failure.

Discussion

Bond strength is defined as the highest force that a material can tolerate prior to fracture. Shear and tensile tests are most commonly used for assessment of bond strength [14]. Polymerization shrinkage of composite creates stress at the adhesive interface [15]. Hydrogen peroxide or its derivatives are used in various concentrations in most bleaching techniques [1]. which produce free radicals such as hydroxyl radicals, superoxide anions, singlet oxygen, and hydroperoxyl [16]. Scanning electron microscopic micrographs have shown that the resin-bleached enamel interface is different from the interface of resin and non-bleached enamel. Resin tags are shallower and defective in bleached enamel [17]; these changes may affect the bond strength and lead to microleakage [18]. Enamel and dentin caries and dentinal tubules may serve as a reservoir for peroxide and free hydrogen radicals [19].

Residual oxygen released from bleaching agents is capable of interfering with adhesive resin infiltration into the etched enamel and prevent complete resin polymerization [1]. Application of antioxidants has been proposed to overcome this problem [2]. Antioxidants

Table 3. Pairwise comparisons of SBS by the Tukey's test

	Groups	Mean Difference(U)	Std. Error	Sig.	95% Confidence	
					Lower	Upper
Control	Standard	12.16764*	0.96143	0.000	9.5906	14.7447
	Pomegranate	-0.08236	0.96143	1.000	-2.6594	2.4947
	Green tea	0.08673	0.96143	1.000	-2.4903	2.6638
Standard bleached	Pomegranate	-12.25000*	0.96143	0.000	-14.8270	-9.6730
	Green tea	-12.08091*	0.96143	0.000	-14.6579	-9.5039
Pomegranate	Green tea	0.16909	0.96143	0.998	-2.4079	2.7461

Table 4. Pairwise comparisons of SBS by the Tukey's test

Group	A	B	C	D
Adhesive	5	7	5	5
Cohesive	1	2	1	2
Mixed	5	2	5	4

neutralize the harmful effects of free radicals [20]. Cavalli et al. [21] showed that at least 3 weeks of delay after bleaching restores the bond strength of bleached enamel to the baseline value. Bulut et al. [22] reported that a minimum of 1-week interval is required between the bleaching and bonding process. According to the literature, immediately after bleaching, the bond strength to enamel declines significantly. According to Sharafeddin et al. [1] immediate application of pomegranate peel, grape seed, or green tea extract following bleaching considerably increases the bond strength of composite to enamel. This method allows us to minimize the number of appointments [1]. Treatment with 10% solution of grape seed extract for 10 minutes following bleaching significantly enhances the bond strength of enamel [8]. This result is similar to that of a previous study that reported effective application of green tea for 1 hour for the same purpose [23]. Mohammadi Bassir et al. [24] reported that treatment with sodium ascorbate and sodium sulfite for 30 minutes after bleaching significantly increased the bond strength to enamel. The green tea applied in this study was made from leaves of Camellia sinensis which is a popular beverage around the world [25]. Epigallocatechin gallate is the most frequent catechin of green tea, which is highly beneficial for the body [10] An animal study on mice well-established that the polyphenols in green tea can reduce the oxidative stress by up to 72% [26]. Also, the green tea polyphenols have free radical scavenging ability. The effectiveness of this compound against hydroxyl radicals and superoxide ions has also been documented [25]. Green tea has no harmful effect on dental structures [10].

Recently, nutritional benefits of pomegranate (Punica granatum) have attracted significant attention because of its high antioxidant activity, attributed to its high content of phenolic compounds. Also, its peel is assumedto contain a higher concentration of polyphenols in comparison with the seeds and pulp [25]. Toxicologically, pomegranate peel extract is safe and non-toxic [27]. Sharafeddin et al. [1] used two different concentrations of pomegranate peel extract (5% and 10%) to neutralize the negative effects of free radicals on the bonding agent polymerization and concluded that the two concentrations were

equally effective on SBS of composite resin to bleached enamel [1]. Thus, we used 5% solution of pomegranate peel extract. It has been demonstrated that improvement of SBS after applying antioxidants is directly proportional to the duration of application [29]. However, other investigations confirmed the effectiveness of 10 minutes of antioxidant treatment and showed that application of antioxidants for more than 10 minutes did not significantly increase the SBS [10,30]. This indicates that 10 minutes of antioxidant treatment is enough to reverse the reduced bond strength and can be easily performed in clinical conditions [23]. We selected sound premolar teeth extracted for orthodontic purposes that belonged to patients of the same age [8]. Single Bond (3M ESPE, USA) was also applied due to its easy availability [1,23]. we applied etchant before the bonding procedure. According to the manufacturer's instructions, application of etchant on the enamel increases the surface area for bonding.

In this study, the effect of antioxidants on SBS of bleached enamel was evaluated and compared. The findings indicated that application of green tea and pomegranate peel extract provided stronger bond compared with immediate bonding (group B). One-way ANOVA revealed a significant difference in SBS of the groups ($P<0.001$). Pairwise comparisons by the Tukey's test showed that immediate application of bonding agent on the bleached enamel (group B) resulted in significant reduction of SBS compared with the control group (group A) ($P<0.001$). These results are consistent with most previous studies [1,4-6, 19]. Also, the present results showed that antioxidant treatment (groups C and D) was significantly effective in increasing the SBS of composite to bleached enamel ($P<0.001$), but there were no significant differences between the groups treated with antioxidants (groups C and D) and the control group (group A) ($P> 0.99$). The results of the present study are in agreement with previous studies [1,22,31]. Khanverdi et al. [10] used epigallocatechin gallate solution on bleached enamel for 20 minutes instead of green tea extract. Although their methodology was different, their results were consistent with the results of the present study. However, Thapa et al. [32] demonstrated that applying α-tocopherol antioxidant on the bleached enamel resulted in no significant difference between the groups, and it was shown to be ineffective. It should be noted that the antioxidant they used was different from the antioxidants assessed in the present study. The information obtained from the bond strength test mostly depends on the actual condition of the test, the geometric shape and surface area of the specimen, and the location and geometry of the loading device that affect stress distribution at the bonded interface and can alter the bond strength [14]. It is difficult to standardize the methodology of bond strength measurement tests. Application of green tea extract antioxidant significantly increased the SBS in the present study ($P<0.001$). This result was consistent with the findings of Sharafeddin study [1]. Also, application of pomegranate peel extract antioxidant significantly increased the SBS of composite to bleach enamel ($P<0.001$). This result was similar to the previously reported findings regarding effective application of antioxidants for the same purpose [1,33] .

According to the current study and previous investigations on this topic [1,22,31], immediate application of antioxidants on bleached teeth is an effective method that significantly increases the SBS of composite to bleached enamel. It was shown to be effective, since there were no significant differences between the groups treated with pomegranate peel and green tea extracts, and the control group. All the antioxidant solutions used in our study were equally effective. Thus, different antioxidants based on their availability can be used to increase the bond strength of composite restoration immediately after bleaching [6,28].

In this study, the dominant failure mode was adhesive; while, cohesive failure was less frequent than other types. This is because the cohesive bond is stronger than the adhesive bond between two dissimilar particles. In group B, with the lowest mean bond strength value, the highest percentage of adhesive failure was observed due to weakening of the enamel surface after bleaching [34,35]. Leloup et al. [34] reported a strong correlation between the bond strength and failure modes, and indicated a higher frequency of mixed failure as the bond strength increased. Our stereomicroscopic findings were consistent with their results. According to Leloup et al, [34] scanning electron microscopic observations of bleached enamel indicated a relationship between high level of voids in the bonding area and low mean bond strength. On the other hand, bubbles were not seen in the groups that were treated with antioxidants; the bubbly appearance of resin may be due to oxygen remaining in the enamel structure. Special attention should be placed on neutralization of oxygen by application of an antioxidant agent.

Conclusion

The findings of the current in vitro study demonstrated that immediate application of antioxidants namely green tea extract and pomegranate peel after bleaching can significantly increase the composite SBS to bleached enamel and neutralize the adverse effects of bleaching. There was no significant difference between different antioxidants in terms of the mean SBS. Further studies could examine other types of available natural antioxidants in order to confirm the optimal efficacy of antioxidants in general for this purpose.

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