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Effect of heating antioxidants and delayed bonding on bond strength to bleached enamel

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***Abstract*---**Objective: The aim of this study was to evaluate the influence of heating natural antioxidants on their antioxidant activity and on micro shear bond strength of composite resin to bleached enamel. Materials and methods. A hundred-eighty flowable composite cylindrical specimens of 0.8 mm diameter and 1 mm length were bonded to enamel substrate for microshear bond strength evaluation. Six study groups were created (n=30). Group 1: No bleaching, Group 2: Bleaching only, Group 3: Bleaching+ Pine Bark Extract at Room temperature, Group 4: Bleaching + Pine Bark Extract preheated to 60° C. Group 5: Bleaching+ Grape Seed Extract at Room temperature, Group 6: Bleaching+ Grape Seed Extract preheated to 60° C. All groups were tested immediately (subgroup a), after one week (subgroup b) and two weeks (subgroup c). Both extracts were tested for their antioxidant activity using DPPH assay at room temperature, 40° and 60°. Two- and one- way ANOVA were used to explore the effect of different surface treatments and bonding time on microshear bond strength. Results. DPPH results showed the heated antioxidant extracts to have significantly higher antioxidant activity than their non-heated counterparts. μSBS results showed that at storage period 2 weeks, Group 1 showed the highest mean values followed by group 4 and group 6. Conclusions. Applying heated antioxidant extracts for 10 min improved the bond strength of composite to bleached enamel.

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Introduction

Vital bleaching has been a viable treatment to discolored teeth, meeting the esthetic standards of contemporary dentistry [1,2]. Chemical bleaching agents like Hydrogen Peroxide (HP) infiltrate both enamel and dentin and go through chemical breakdown forming oxygen free radicals. The benefit of these free radicals is that they are highly unstable and capable of oxidizing and disintegrating pigments macromolecules producing the desirable "whitening effect" [3]. However, they have the disadvantage of being highly reactive that they also oxidize the free radicals generated by polymerization initiators, rendering them unable to fully react with the dental composite monomers which decrease the polymerization reaction. This combined with the reduction in the Calcium/Phosphorus content of the enamel after bleaching, leads to lower shear bond strengths (SBS) of the post-bleaching composite restorations. Compromised composite/enamel bond strength adversely affects the final restoration physio-mechanical properties and affects its longevity [3].

Eliminating this free radicals-filled layer leads to the enhancement of SBS of bleached enamel to composite resin [2]. Many approaches have been proposed to counteract the effect of the bleaching agents on the composite bond strength, namely being alcohol treatment of bleached enamel, utilization of organic-solvent-containing adhesives, removing the outermost layer of enamel, and the postponement of the bonding procedure for a period ranging from 24 hours to four weeks after bleaching; however, most of these approaches are not clinically feasible in most cases [4, 5].

Using antioxidant extacts has recently presented promising results in reversing the temporary negative effects caused during chemical bleaching. Grape seed extract (GSE) and pine bark extract (PBE) are two of the most used natural antioxidant agents which contain oligomeric proanthocyanidin complexes (OPCs) which are a class of polyphenolic bioflavonoids characterized by their antibacterial, antiviral, anti-inflammatory, anticarcinogenic, as well as their free radical scavenging ability, which allows the adhesive to continue its polymerization without premature interruption thus leading to improved bonding [2, 5].

Many antioxidative polyphenolic compounds are naturally present in a covalently bound insoluble polymeric form; however, they are affected by simple heat treatments that induce their breakdown and conversion to low-molecular-weight monomeric forms. These simpler forms have increased amounts of phenolics and active compounds as well as a raised antioxidant activity [6][7][8][9].

DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) free radical method is an antioxidant assay based on electron-transfer that produces a violet solution in ethanol. This free radical, which is stable at room temperature, is reduced in the presence of an antioxidant molecule, giving rise to colorless ethanol solution. The use of DPPH assay provides a fast and simple way to measure the free-radical

scavenging ability of any compound, and it evaluates the antioxidant activity by spectrophotometry, so it can be useful to assess various products at one time [10].
The aim of this study is to assess the effect of preheated grape seed extract (GSE) and pine bark extract (PBE) as well as delayed bonding on shear bond strength between composite resin and bleached enamel. The first null hypothesis was that preheating of grape seed and pine bark extracts has no effect on their antioxidant activity. The second null hypothesis was that preheating of grape seed and pine bark extracts as well as–delayed bonding has no effect on microshear bond strength of composite resin to bleached enamel.

Materials and methods

Preparation of Antioxidants

Ten grams of grape seed extract (Puritan's pride, Holbrook, NY, USA, lot no. 17030B) and pine bark extract (Puritan's pride, Holbrook, NY, USA, lot no. 7K033) were dissolved in 100 ml of distilled water each to make 10% w/v solution. Half of each prepared solution was heated in a hot water bath until the temperature reached 60° C and then left to cool down back to room temperature [2].

DPPH assay

Each extract was weighed 5 mg of the extract accurately, dissolved in 10 ml methanol and divided to three groups (n=3); Group A: Extract kept at room temp, Group B: the extract was heated at 40 C° for 10 min then cooled to room temperature, Group C: the extract was heated at 60 C° for 10 min then cooled to room temperature [11]. Then, eight test tubes were taken to make aliquots of 8 conc. (5, 10, 15, 20, 25, 30, 35 and 40 µg/mL) with each group using dilution by methanol. DPPH was weighed and dissolved in methanol to make 0.004% (w/v) solution. After then, 3 ml of 0.004%(w/v) DPPH solution was applied by pipette on 40 µL of each test tube and methanol (as blank). After 30 min, the absorbance of each test tube was taken by a UV spectrophotometer at 517 nm. Ascorbic acid was used as standard [12].

The results were calculated using the following equation and expressed as the IC_{50} (μg/mL) values that represent the antioxidant concentration able to scavenge 50% of DPPH radical present. Such test was done triplicate for both GSE and PBE.

DPPH radical scavenging activity = $[(A_0–A_1)/A_0] \times 100$

A_0 was the absorbance of the control and A_1 was the absorbance of the sample.

Shear bond strength test:

Specimen preparation:

Forty-five sound human lower wisdom teeth (disinfected with thymol 0.1 % for 30 days after extraction) were washed by distilled water then cleaned by ultrasonic scaler and then examined to be free of any cracks or caries. Each crown was then sectioned mesiodistally into two halves, using a precision diamond saw (IsoMet 4000; Buehler, USA) while the roots were sectioned transversally 2 mm below the cementoenamel junction, producing 90 coronal halves. Each coronal half was fixed in acrylic resin with its labial surface facing upwards [13]. The center of each embedded coronal half was flattened with a 600-grit silicon carbide paper for 60 seconds to create a smooth, flat enamel surface for treatment and bonding of composite resin [3].

Specimen grouping:

A total of 180 composite specimens were randomly allocated according to surface treatment and bonding time into six groups (n=30):
Group 1: No Bleaching (Negative Control) (NC)
Group 2: Bleaching only (positive control) (PC)
Group 3: Bleaching+ Pine Bark Extract-Room Temp (B+PBE-R)
Group 4: Bleaching+ Pine Bark Extract–60° C (B+PBE-60)
Group 5: Bleaching+ Grape Seed Extract– Room Temp (B+GSE-R)
Group 6: Bleaching+ Grape Seed Extract– 60° C (B+GSE-60)
All groups were tested immediately (Subgroup a), after one week (Subgroup b) and two weeks (Subgroup c).

Bleaching Procedure

The Opalescence Boost PF 40 % (Ultradent product, South Jordan, UT, USA) was activated chemically with syringe-to-syringe mixing process according to the manufacturer instructions. A 1 mm thick layer of bleaching gel was applied on enamel surface for 20 min for three consecutive cycles. After each cycle, teeth were thoroughly rinsed with water for 60 seconds [14] and air-dried for 30 seconds [15]. After bleaching and before testing, samples in all subgroups (b) were stored for one week and samples in all subgroups (c) were stored for two weeks, all in distilled water in an incubator at 37°C. The rest of the samples in subgroups (a) were passed on to be tested immediately.

Application of antioxidants and Bonding Procedures

No antioxidant solutions were added to the samples of groups I and II. Using a syringe, a volume of 1 ml of each of PBE and preheated PBE solutions were applied to group III and group IV respectively for 10 min [2]. Both GSE and preheated GSE solutions were applied to group V group VI respectively for 10 min [2]. The surfaces were then rinsed with distilled water for 30 seconds and dried using compressed air for 5 seconds [15].

The enamel was etched with 37% phosphoric acid (Meta Etchant, Meta biomed, Korea, Lot No. MET1906071) for 30 seconds, then rinsed thoroughly for 15 seconds with distilled water and air dried for 10 seconds [16]. Total-etch adhesive

(Adper single bond 2, 3M ESPE, Saint Paul, MN, USA, Lot No. N881328) was applied actively to the polished enamel surface, followed by gentle air jet for 10 seconds according to manufacturer's instructions. Two tubes were placed over the adhesive coated enamel surface before light curing [13]. The adhesive was light cured for 20 seconds using 1S LED curing unit light (CICADA Dental Instruments Co. Ltd., Guangdong, China) [14]. Flowable composite (3M ESPE Filtek Z350 XT St. Paul, MN, USA, Lot No. N902077) was injected into the silicone tubes from bottom upwards to avoid bubbles inclusion then light cured for 40 seconds according to manufacturer instructions [14]. Silicon tubes were then vertically sectioned carefully with a sharp lancet giving two composite stubs bonded to the enamel. Specimens were then stored in distilled water at 37 °C in an incubator for 24 hours until testing [13].

Micro SBS Test

The μSBS was performed using universal testing machine (Instron model 3345, England) with cross head speed of 0.5 mm/min using wire loop driving the load onto the specimen at the enamel- composite interface till specimen debonding [13].

After debonding, the diameter of each cylindrical composite stub was measured with a digital caliper to identify any diameter variabilities from the silicon mold. μSBS was recalculated according to the measured diameter of the specimen using the following equation: $\boldsymbol{\sigma = F/A}$

where: σ is microshear bond strength in Mega Pascals (MPa). F is failure load in Newtons (N). A is surface area in square millimeters (mm^2).

Statistical analysis

According to an internal pilot study, sample size was calculated using G*Power version 3.1.9.2 for sample size analysis at α=0.05 and 95% power and effect size equal to 0.75524 which yields a sample size of 7 samples per group. Ten samples (n=10) per group were performed to gain extra power. Statistical analysis was computed using SPSS (statistical package for social sciences, IBM SPSS Statistics for mac, version 24 software, Armonk, NY: IBM Corp, USA). Data were presented as means and standard deviations. Data were checked for normality using Kolmogorov – Smirnov test and Shapiro- test and were found to be normally distributed. Following significant interactions, One-way between-groups analysis of variance (ANOVA) was conducted to further explore the effect of different antioxidant surface treatment on μSBS for each bonding time. One-way between-groups analysis of variance (ANOVA) was conducted to further explore the effect of different time on μSBS for every antioxidant surface treatment. Post-hoc comparisons using the Tukey's test was used to investigate differences between groups and significance level was set at ($p \leq 0.05$).

One-way between-groups analysis of variance (ANOVA) was carried out to compare the antioxidant effect of different antioxidant surface treatments. Post-hoc comparisons using the Tukey's test was used to investigate differences between groups and significance level was set at ($p \leq 0.05$).

Results

DPPH Assay Results

One-Way ANOVA results, summarized in Table (1) showed that (GSE 60°) and (GSE 40°) have the highest antioxidant activity which was statistically significant from GSE at room temperature. They were followed by (PBE 60°) and (PBE 40°) which was statistically significant from PBE at room temperature.

Table (1): Mean and standard deviation (SD) values for different antioxidant IC_{50} (µg/ml)

Antioxidant (Temp)	**Mean**	**Std. Deviation**	**p value**
Ascorbic acid reference	13.98[a]	0.20	0.0001
Pine Bark Extract (Room temp)	6.75[b]	0.07	
Pine Bark Extract (40 °C)	6.14[c]	0.25	
Pine Park Extract (60 °C)	5.82[cd]	0.22	
Grape Seed Extract (Room temp)	5.50[d]	0.10	
Grape Seed Extract (40 °C)	4.47[e]	0.15	
Grape Seed Extract (60 °C)	4.35[e]	0.26	

Different **small letters indicate significant difference within the same **column**.*

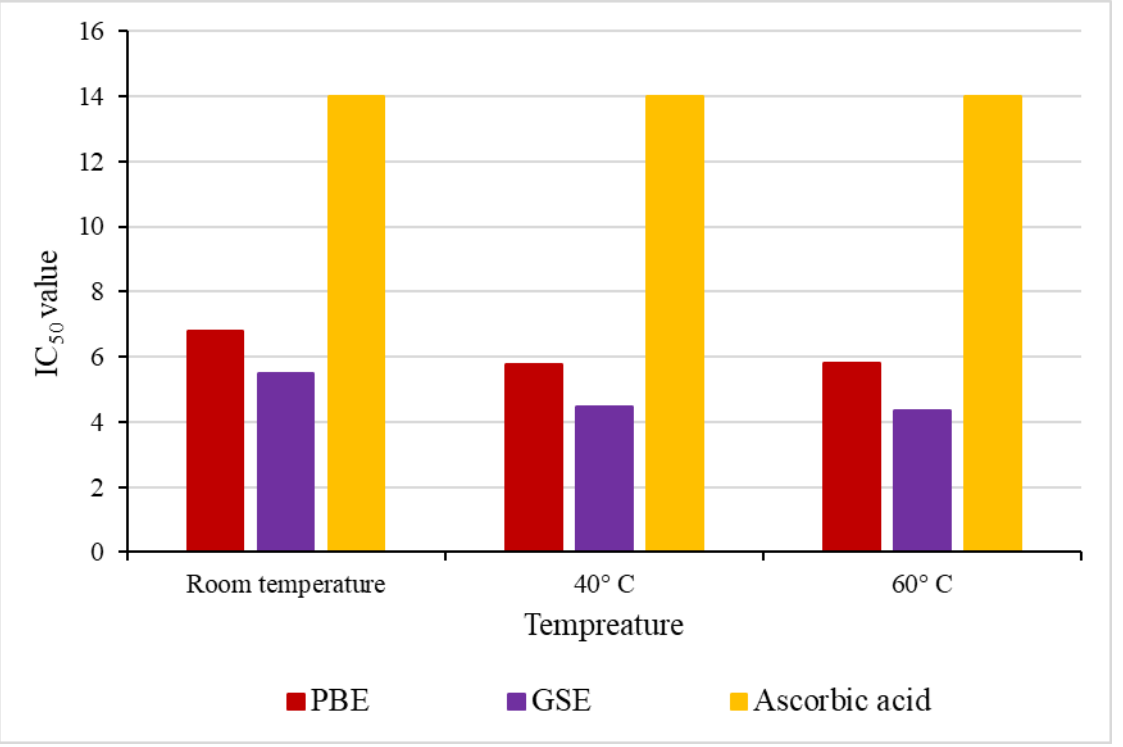

Figure (1): Bar chart showing mean values of IC_{50} (µg/ml) of different antioxidants at different temperatures

Microshear Bond Strength Results:

Microshear bond strength results are illustrated as means and standard deviations in Table (2) Regarding immediate bonding, Group 1 showed the highest mean value for µSBS followed by group 4 and group 6 with no statistically significant difference between the latter two groups. These were followed by group 3, and group 5 with no statistically significant difference between these two groups. Group 2 showed the lowest mean value.

Table 2: One-way ANOVA results showing the effect of surface treatment and time on mean shear bond strength

Surface treatment	Time of bonding			P-value
	Immediate	1 week	2 weeks	
	Microshear bond strength Mean (SD)			
GSE-R	12.92(4.32)^{cC}	21.36(3.82)^{bB}	29.55(4.54)^{bA}	0.001
GSE-60°	19.18(1.27)^{bC}	32.50(6.63)^{aB}	37.86(2.11)^{aA}	0.001
PBE-R	12.48(3.39)^{cC}	20.51(3.71)^{bB}	25.76(5.32)^{bA}	0.001

PBE-60°	19.23(1.92)^{bC}	30.37(4.13)^{aB}	36.91(5.87)^{aA}	0.001
PC	6.33(1.24)^{dC}	15.86(4.65)^{bB}	21.38(5.69)^{bA}	0.001
NC	40.42(16.3)^{aA}	40.88(18.07)^{aA}	41.93(17.47)^{aA}	0.335
P-value	0.001	0.001	0.001	

Different **small letters indicate significant difference within the same **column** for every storage time.*

Different **Capital letters indicate significant difference within the same **row** for every surface treatment.*

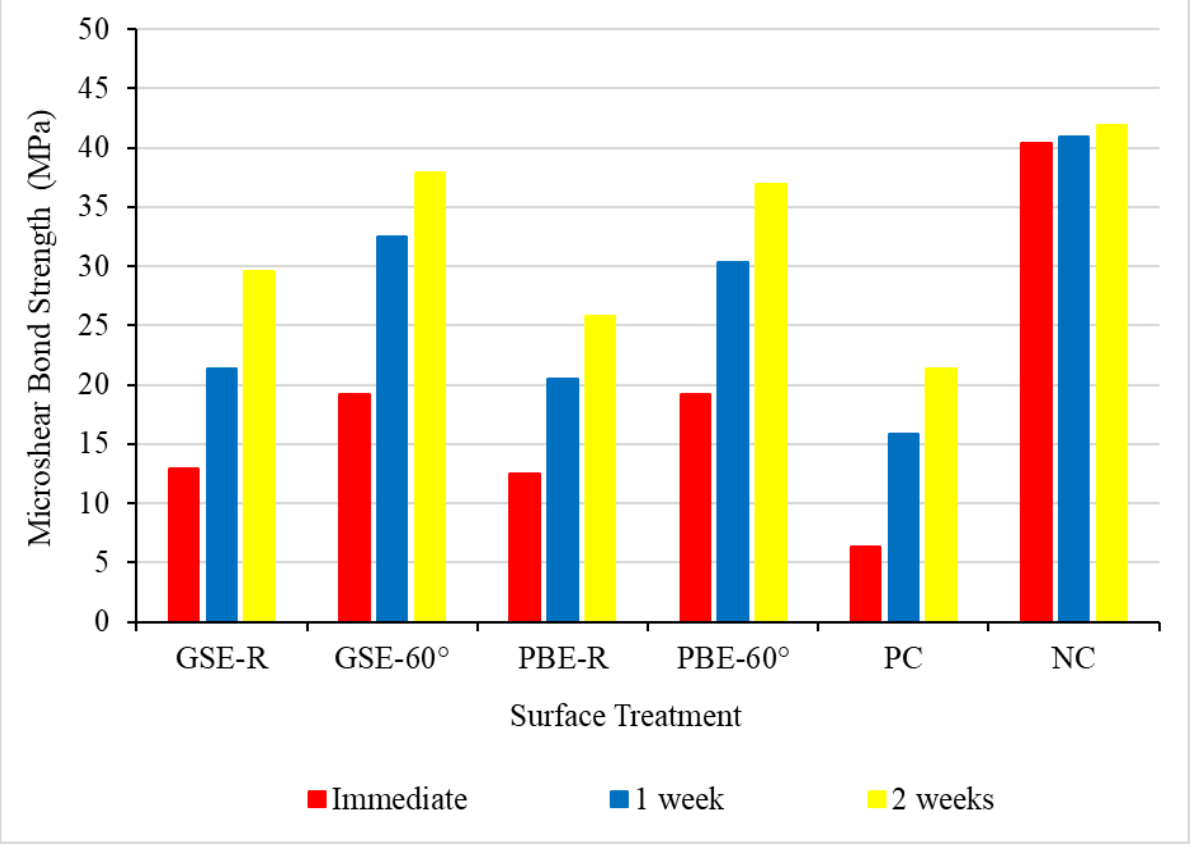

Figure (2): Bar chart showing mean values of micro-shear bond strength (MPa) for different enamel surface treatment groups.

After 1-week delayed bonding, there was no significant difference between group 1 and each of group 4 and group 6 which showed the highest μSBS values. Followed by group 3, and group 5, and the group 2 showed the lowest mean value. There was no significant difference between group 2 and each of group 3 and 5. Then Group 6 is statistically higher than group 5 as well as group 4 has higher mean value than group 3.

After 2-weeks, there was no significant difference between group 1 and each of group 4 and group 6 which showed the highest μSBS values followed by group 3, and group 5. Group 2 showed the lowest mean value. There was no significant difference between group 2 and each of group 3 and 5. Then Group 6 is statistically higher than group 5 as well as group 4 has higher mean value than group 3.

Regarding the effect of bonding time on μSBS, for the group 1, there was no significant difference between the 2-week, 1-week, and immediate groups. For all other surface treatments, there was a statistically significant difference between all the three tested groups. with two weeks bonding showing highest μSBS while immediate group showed lowest μSBS.

Discussion

Bleaching agents were proven to cause morphological alterations of teeth, increased surface irregularities, roughness, and reduced microhardness of enamel in addition to decreased bond strength of composite resin to enamel [4].
The compromised bond strength following bleaching is due to the effect of bleaching agent leaving behind a residual oxygen layer which interferes with the resin infiltration into etched enamel and inhibits the polymerization of resin, this is proven by our study that the untreated bleached enamel showed the lowest μSBS values in all testing times [1] [3] [4].

To overcome this problem, the treatment of the bleached dental enamel with natural antioxidants has been recommended in recent years. GSE and PBE are considered the most potent natural antioxidants as they contain 98% proanthocyanidin which is a class of polyphenolic bioflavonoids [2].

It is claimed that the proper time for antioxidant use is 10 min which is enough for reversing the compromised bond strength, where any further increase in time of application will cause no meaningful increase in μSBS. The antioxidant concentration used in this study was 10% w/v, any concentrations lower than that were found to be not as reliable in reversing compromised bond strength [17].

The antioxidant activity of GSE and PBE can be attributed to the multiple donor sites found on its OPC which have a specificity for trapping the superoxide and hydroxyl free radicals which enhances free radical scavenging and increases the bond strength of composite to bleached enamel [18].

Preheating of both GSE and PBE for bleached enamel surface treatment was done up to 60 °C. Such temperature was chosen based on the results of the antioxidant activity (DPPH assay) results which showed that preheating of both antioxidants (either to 40° or 60°) produced a significantly higher antioxidant effect than using them in the non-preheated form, thus rejecting the first null hypothesis. However, preheating to 60 °C was chosen rather than the 40 °C depending on conclusions of previous studies [19] [20] [21].

DPPH assay also showed that GSE had higher antioxidant activity than PBE. This could be attributed to their different phenolic compositions. However, such difference was not reflected on the μSBS values, where no significant difference was detected between the corresponding GSE and PBE groups. Previous studies have also shown both extracts to exhibit a similar antioxidant effect potent enough to completely negate the effect of the bleaching gel on enamel bond strength [2].

Surface treatment of bleached enamel using preheated GSE and PBE to 60° C showed a significantly higher μSBS values compared to using non-preheated extracts in all the testing times. This can be explained by the fact that preheating GSE and PBE increases the total phenolic content (TPC) by inactivating polyphenol oxidase enzyme which inhibits polyphenolics degradation as well as allowing bound flavonoid/phenolic compounds to be liberated which overall increases the amount of free active low-molecular-weight antioxidant compounds in the extract [8][19]. These findings were further supported by the DPPH assay results.

Regarding one-week and two-week delayed bonding, the groups treated by both preheated antioxidant extracts were able to completely restore the enamel bond strength up to the level of the natural non-bleached group (negative control). Previous studies have also shown one-week delayed bonding to be sufficient to enhance the enamel bond strength [17] [22]. While others showed that three weeks may be needed to achieve the same effect [2]. These results have also revealed that the groups treated with non-preheated antioxidant extracts had μSBS values with no significant difference to that of the bleached non-treated group (positive control). All of this further reiterate the role of delayed bonding in reversing the compromised enamel bond strength. Based on the previous findings the second null hypothesis was rejected.

Conclusion:

Within the limitations of this study, we can conclude that:
- Surface treatment of bleached enamel with either GSE or PBE failed to restore immediate bond strength to bleached enamel.
- Preheating GSE and PBE to both 40° C and 60° C enhanced their antioxidant effect.
- Preheating GSE and PBE to 60° C enhanced their effect on restoring the μSBS of bleached enamel to composite.
- Using the delayed bonding strategy alone improved the μSBS but was not potent enough to restore μSBS to bleached enamel back to the non-bleached condition.
-Delaying bonding to bleached enamel for one week is sufficient to restore the bond strength if enamel surface is treated with preheated GSE or PBE.

Limitations of the study:

Time of application of antioxidant, discoloring effect caused by GSE and PBE application.

Recommendation:

Reduce the time of delayed bonding and time of antioxidant application. Measuring color change is recommended.

Conflict of interest:

The authors report no conflict of interest. The authors have no proprietary, financial, or other personal interest of any nature or kind in any product, service, and/or company that is presented in this article.

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