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Comparative Evaluation of Two Antioxidants on Reversing the Immediate Bond Strength of Bleached Enamel: *In Vitro* Study

Authors' Contribution:

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Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
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Background: Tooth bleaching causes a significant decrease in the bonding strength between the resin and human enamel. Nevertheless, the effects of different antioxidant types on the immediate bonding strength of resin and bleached enamel were significantly different. Therefore, the objective of this study was to compare the effects of 2 antioxidants for enhancing the bond strength of the resin to bleached enamel.

Material/Methods: There were 48 enamel blocks performed from 48 recently extracted maxillary central incisors. There were 8 groups: NC (negative control, no bleached specimens restored without antioxidants); NA (no antioxidant, bleached specimens bonded immediately without any antioxidants); SA30, SA60, and SA120 (bleached specimens accepted the management of 10% sodium ascorbate (SA) for 30 minutes, 60 minutes, and 120 minutes, respectively, before restored); PC30, PC60, and PC120 (bleached specimens received treatment of 5% proanthocyanidins (PC) for 30 minutes, 60 minutes, and 120 minutes, respectively, before restored). We measured the micro-tensile bond strength of specimens and used 2-way ANOVA to analyze the data.

Results: The mean±standard deviation bond strength measured were: NC, 29.99±4.00; NA, 14.90±1.97; SA30, 18.60±2.20; SA60, 22.57±2.71; SA120, 26.15±3.85; PC30, 16.78±2.29; PC60, 19.13±2.24, PC120, 23.90±2.01 MPa. In addition, the fracture types were mainly of an adhesive mode (88.75%), followed by mixed (7.5%), and cohesive (3.75%).

Conclusions: 10% sodium ascorbate provided a comparatively more promising improvement for immediate bond strength than 5% proanthocyanidins when the same duration of antioxidant was applied.

MeSH Keywords: **Adhesives • Antioxidants • Bleaching Agents • Proanthocyanidins**

Abbreviations: **SA** – sodium ascorbate; **PC** – proanthocyanidins; **MTBS** – micro-tensile bond strength; **MSBS** – micro-shear bond strength; **ANOVA** – analysis of variance; **SEM** – stereomicroscope; **BS-RBE** – bond strength of resin to bleached enamel

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Background

With the improvement of people's aesthetic concept, more and more people have received bleaching treatment of discolored teeth [1–3]. However, scholars reported that tooth bleaching could decrease the bond strength of resin to bleached enamel (BS-RBE), primarily when bonding is conducted at once after the tooth bleaching [1–23].

Delaying the bonding time is a common method to improve the BS-RBE, with the waiting period ranging from 1 day to 28 days [9,24–26]. However, sometimes, cases are too urgent to wait for their teeth restoration [23]. In order to prevent the delay of recovery treatment after bleaching, various antioxidant agents, including sodium ascorbate (SA) [16,21,27], proanthocyanidins (PC) [28,29], green tea extract [4,5,7,13,16], pine bark extract [30], aloe extract [5,16], and grape seed extract [16,31], have been attempted, among which, SA and PC have shown promising results [4,5,14,28,29]. Nevertheless, the results of antioxidants with different types and durations are quite different [4,5,8–10,32,33]. Consequently, we conducted the present *in vitro* study to compare the effects of 10% SA and 5% PC on the immediate BS-RBE.

Material and Methods

The study was approved by the ethics committee of the Stomatological Hospital of Jilin University and signed the informed consent document.

Dental specimen preparation

Forty-eight intact incisors were collected, not including caries, tetracycline, and dental fluorosis (Oral and Maxillofacial Surgery Clinic Office of Stomatological Hospital of Jilin University, China). An enamel slab specimen with 5×5 mm made from maxillary central incisors was extracted within 1 month by using a dental high-speed turbine handpiece at low speed under water irrigation. The surface of all specimens was polished to #1000 by silicon carbide abrasive paper (Hubei poses with Abrasive Belt Group Co., Ltd., China) under water irrigation to obtain a 5 mm² flat enamel area. Crack-free teeth in crowns were identified under a stereomicroscope (SEM), which were then stored in a 1% chloramine T solution (Tianjin Guangfu Fine Chemical Research Institute, China) at 4°C.

Soft tissue on the surface of specimens was removed by ultrasonic vibration cleaning. Then, 48 samples were averagely and randomly categorized as the following 8 groups. NC (negative control): specimens were bonded without bleaching; NA (no antioxidant): bleached specimens were restored immediately without any antioxidant treatment; SA30, SA60, and

SA120: bleached specimens received the management of 10% SA (Beijing Solebo Technology Co., Ltd. China) before restoration for 30 minutes, 60 minutes, and 120 minutes, respectively; PC30, PC60, and PC120: bleached specimens were treated with 5% PC (Shanghai Yuanye biological Co., Ltd. China) before restoration for 30 minutes, 60 minutes and 120 minutes, respectively.

Bleaching procedure

All specimens in the 8 groups were bleached by utilizing 35% hydrogen peroxide (VIVA Technology International Co., Ltd., USA) on the surface of enamel with a 1 to 2 mm thin layer, with the light of oral whitening lamp as close as possible perpendicularly illuminating the surface of the enamel. The bleaching agent was removed after 10 minutes and then reapplied. Specimens were bleached for 3 times for 30 minutes in total, 10 minutes each time. Specimens, after each bleaching, were rinsed for 1 minute, wiped by a cotton ball, and dried by blowing machine (Shanghai Jinghong Experimental Equipment Co., Ltd. China).

Treatment with antioxidants

Bleached specimens assigned to SA30, SA60, and SA120, were exposed to 10% SA gel for 30, 60, and 120 minutes, respectively, while those in PC30, PC60, and PC120 were exposed to 5% PC for 30, 60, and 120 minutes, respectively. After antioxidant treatment, the enamel surface was rinsed using saline irrigation for 30 seconds (Basic Laboratory, School of Stomatology, Jilin University, China).

Restoration with composite resin

Each prepared specimen was etched by 37% phosphoric acid for the 20 seconds, rinsed for 30 seconds, followed by an air dry for 20 seconds. Modified acrylate adhesive (GE LiaHao New Material Co., Ltd., China) was applied over the etched enamel and then spread with a gentle wind. A transparent polypropylene tube of 4 mm x 4 mm in size was placed in the central area of enamel to limit the bonding range, and then light-cured for 20 seconds. The resin matrix was divided into 3 layers for stacking up to the height of the tube orifice, with 40 seconds each time. The bonded specimens were stored for 24 hours in normal saline at room temperature, and then were embedded in transparent epoxy resin (Shenzhen Hengda Technology Co., Ltd., China) to prevent fracture during cutting.

Micro tensile testing

The specimens were cut into 24 small strip sticks with a size of 1×1×8 mm (Figure 1) using a low speed cutting machine (Shenyang Kejing Automation Equipment Co., Ltd., China) underwater washing. Ten sticks were randomly chosen and

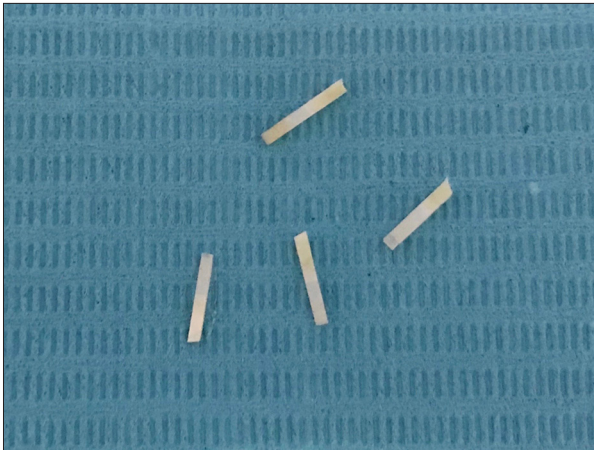


Figure 1. Sticks, performed with the size of 1×1×8 mm, were prepared for micro-tensile bond strength (MTBS) testing.

confirmed to be defect-free and crack-free through SEM for micro-tensile bond strength (MTBS) testing.

A universal test machine (Shimadzu Corporation, Japan) (Figure 2) was used to measure MTBS by stretching at a speed of 1 mm/minute, and the SEM was utilized to identify the fracture types. The micrometer (Guilin Measuring Tools and Cutting Tools Factory, China) was used to calculate the actual bond area of the specimens, and the MTBS was conducted according to the formula of $MTBS = F/m^2$.

The sticks were polished from teeth surface to resin matrix surface with 600, 800, and 1000 mesh silicon carbide water sandpaper, respectively, under irrigation. SEM was used to observe the surfaces of the sticks. The measured surfaces were treated with 37% phosphoric acid etchant for 3 seconds, then rinsed with saline and dried by an air compressor, and then the gradient dehydration of sticks was conducted in 50%, 70%, 90%, and 100% ethanol for 10 minutes, respectively. The sticks were dried in a blast drying chamber at 50°C for 4 hours and then fixed on the loading table. Finally, vacuum gold spraying coating was prepared by ion sputtering, along with the observation of the micro-morphology of bonding interface under scanning electron microscopy.

Statistical analysis

Statistical analysis of the MTBS values of the 8 groups was conducted by SPSS version 24 (IBM Corp., USA) through carrying out a 2-way ANOVA test. Shapiro-Wilk was applied to test the normality of data, and Levene was used to checking the homogeneity of variance. Multiple comparisons of the Tukey test were made at different treatment times of 10% SA solution and 5% PC solution ($P < 0.05$).

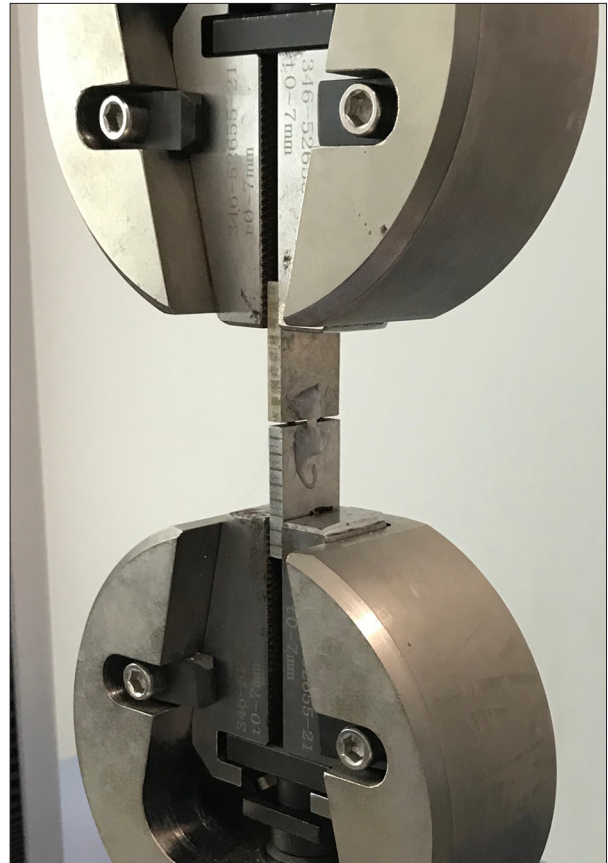


Figure 2. Micro-tensile bond strength (MTBS) testing was conducted at 1 mm/minute using a universal testing machine.

Results

Commonly, the fracture types were classified as adhesive fracture, cohesive fracture, and mixed fracture, according to El Zohairy et al. [34]. To be more specific, the adhesive fracture was defined as fracture occurring between enamel and resin, and the cohesive fracture was regarded as the only enamel substrate failure, and the mixed fracture was viewed as the coexistence of enamel substrate and resin materials fractures. In addition, the mean±standard deviation (SD) bond strength measured as follows: NC, 29.99±4.00; NA, 14.90±1.97; SA30, 18.60±2.20; SA60, 22.57±2.71; SA120, 26.15±3.85; PC30, 16.78±2.29; PC60, 19.13±2.24, PC120, 23.90±2.01 MPa (Figure 3).

Two-way ANOVA variance analysis demonstrated that the duration, as well as type of antioxidant treatment, exerted significant impacts on the MTBS ($P < 0.05$), without the interaction between the types of antioxidants and the duration of therapy on the MTBS ($P > 0.05$). In other words, a more BS-RBE was observed in 10% SA than 5% PC ($P < 0.05$). Besides, extending the use time of antioxidants can improve the MTBS significantly ($P < 0.05$).

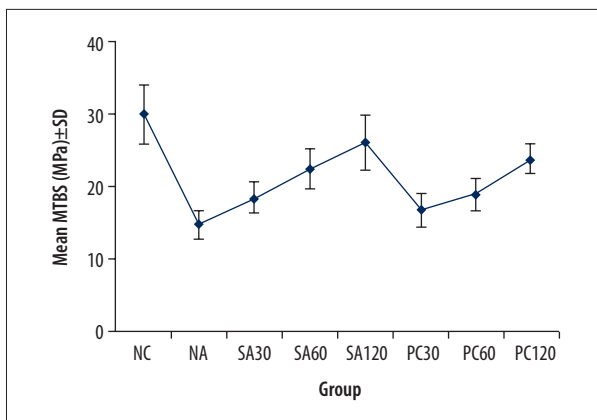


Figure 3. The mean value of micro-tensile bond strength in different groups was measured, with micro-tensile bond strength (MTBS) in figure representing micro-tensile bond strength.

Tukey, multiple comparison test results suggested that the MTBS of specimens after 10% SA treatment for 30, 60, and 120 minutes were of significant difference ($P < 0.05$). Meanwhile, it had no significant difference when treated with 5% PC treatment for 30 and 60 minutes ($P > 0.05$), while it had a substantial difference when treated with 120 minutes ($P < 0.05$).

The fracture types, as listed in Table 1, were also detected. SEM showed that fracture types (Figure 4) mainly showed an adhesive mode (88.75%), followed by mixed (7.5%), and cohesive (3.75%) mode.

Discussion

Tooth bleaching is a cosmetic method to change the color of tooth structure through bleaching agent [5,7–9,12,18]. It is advantageous due to its harmlessness to the tooth and

surrounding tissues, simple operation, safety, and quick effect. To date, bleaching agents, such as hydrogen peroxide with concentration from 30% to 40% or carbamide peroxide with level from 35% to 37%, have been applied in clinics successfully, getting satisfying aesthetic results [3,4,6,8–10,15,35]. However, most scholars suggested tooth bleaching can decrease the immediate BS-RBE [4–9,15,36]. It is due to bleaching agents damage the surface morphology of enamel. Besides, hydrogen peroxide is decomposed into free radicals and oxygen, which interfere with resin penetration and prevent polymerization of the resin [6,21,37]. Various methods to reverse the decrease of BS-RBE are available, including antioxidant treatment, ethanol pretreatment of enamel surface after bleaching before resin bonding, the utilization of an adhesive containing an organic solvent, and the removal of the enamel surface [14,27,38,39]. However, the removal of surface enamel is disadvantageous in increasing the number of teeth prepared and affecting the pulp status. Ethanol pretreatment of the enamel surface cannot restore the BS-RBE completely, making it still necessary to be combined with postponed rehabilitation. To date, literatures demonstrated that the postponing of 7 to 28 days after enamel bleaching before resin bonding is an effective method [8,9,14,24]. Nevertheless, it increases the number of office visits, thus bringing great inconvenience to patients [23]. In recent years, antioxidant treatment has provided us with promising results to reverse the decline of BS-RBE [3-12,14,19,23,40]. Currently, a variety of antioxidants are used to enhance BS-RBE. However, there is no study comparing the effect of 10% SA and 5% PC on the immediate BS-RBE. Therefore, the purpose of this *in vitro* comparative study was to estimate the impact exerted by 10% SA and 5% PC on immediate BS-RBE.

Bleached group versus unbleached group

In our study, we found that the bleached groups (NA, SA30, SA60, SA120, PC30, PC60, and PC120) have lower MTBS than

Table 1. Fracture modes found in the experimental groups, N (%).

Group	Adhesive fractures	Cohesive fractures	Mix fractures	Total number of sticks
1	7 (8.75%)	2 (2.5%)	1 (1.25%)	10
2	9 (11.25%)	0 (0.00%)	1 (1.25%)	10
3	9 (11.25%)	0 (0.00%)	1 (1.25%)	10
4	9 (11.25%)	0 (0.00%)	1 (1.25%)	10
5	9 (11.25%)	0 (0.00%)	1 (1.25%)	10
6	9 (11.25%)	1 (1.25)	0 (0.00%)	10
7	10 (12.50%)	0 (0.00%)	0 (0.00%)	10
8	9 (11.25%)	0 (0.00%)	1 (1.25%)	10
Total	71 (88.75%)	3 (3.75%)	6 (7.5%)	80

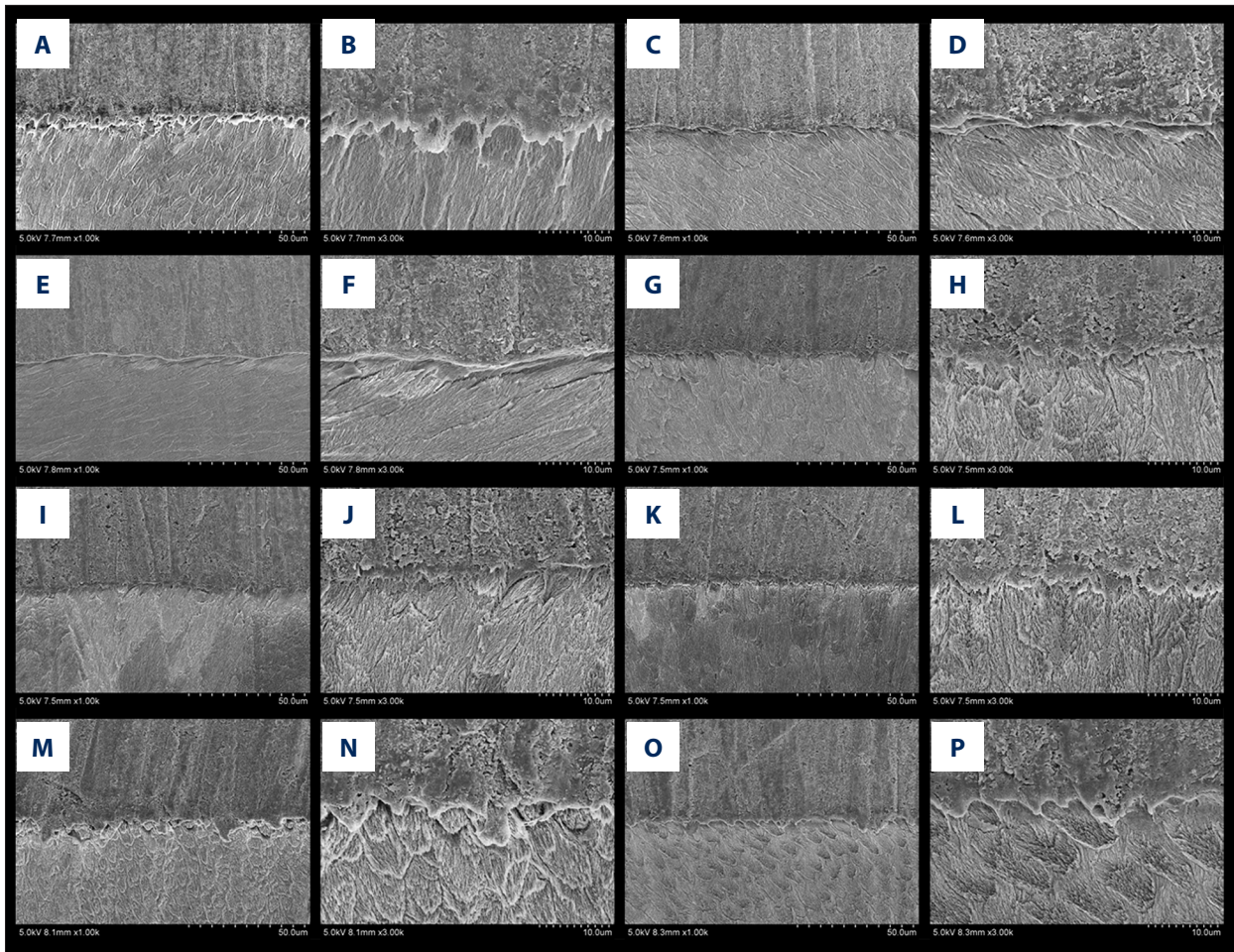


Figure 4. Scanning electron microscopy pictures after bonding tests of all groups. (A, B) The negative control (NC) group showed that the resin-enamel interface was compact, without crack, along with the closely chimeric resin process and enamel. (C, D) The no antioxidant (NA) group demonstrated that the interface between enamel and resin cement was loose and cracked. (E, F) The sodium ascorbate at 30 minutes (SA30) showed that the interface of group 3 was denser than group 2, but there were still cracks. (G, H) The sodium ascorbate at 60 minutes (SA60) group and (I, J) the sodium ascorbate at 120 minutes (SA120) illustrated that the interface between enamel and resin was compact and crack-free, along with chimeric the resin process and enamel. (K, L) The proanthocyanidins at 30 minutes (PC30), and (M, N) the proanthocyanidins at 60 minutes (PC60) showed that the interface between enamel and resin was clear, but the band was loose. There were scattered cracks resin protrusions. (O, P) The proanthocyanidins at 120 minutes (PC120) showed that the interface between enamel and resin cement was not clear, along with the solid bond, without crack. The formation of resin protrusion could be observed.

the unbleached group (NC group). Gonzalez-Lopez S et al. [35] reported that bleaching changed the composition of organic in enamel, and decreased the content of phosphate and carbonate, thus making the enamel surface rough and irregular. Our experimental results are consistent with the conclusions drawn by Gonzalez-Lopez et al. [35]. Besides, scholars also suggested that different concentrations, duration of application, and pH value of bleaching agents may cause different severities of damage to the enamel surface. Wang et al. [20] found that prolonged bleaching time could aggravate the destruction of enamel surface morphology. Besides, several studies have shown that a low pH bleaching agent can worsen enamel demineralization [22]. Therefore, the mild hydrogen peroxide with

a concentration of 35% was chosen in our experiment, with a total bleaching time of 30 minutes, to minimize the damage to the enamel surface.

Antioxidant group versus non-antioxidant group

According to the previous studies, the BS-RBE with antioxidants was significantly higher than that without [12–14]. Furthermore, De Carvalho HC et al. [12] found the most useful BS-RBE when the concentration of SA was 10%. However, the BS-RBE decreased with the concentration increasing to 20% and 30%. Meanwhile, PC is an antioxidant with vigorous antioxidant activity as well as a water-soluble natural, free

radical scavenger, almost without side effects [28,29,32,33]. It was applied successfully in many dental treatments. Thus, SA with 10% concentration and 5% PC was chosen in this experiment. In our study, the specimens of the NA group which received the resin bond immediately after bleaching, without receiving any antioxidant treatment, demonstrated the lowest MTBS values at the resin-enamel interface. We argue that this result could be attributed to residual oxygen produced by hydrogen peroxide on the superficial enamel layer, which prevents the polymerization of the bonding agents. This view was in line with what is proposed in literatures [9,18,21,31].

10% SA group versus 5% PC group

Both 10% SA and 5% PC treated with bleached enamel for 120 minutes could achieve satisfying composite bond strength with 26.15 ± 3.85 MPa and 23.90 ± 2.01 MPa, respectively, which were higher than clinical restoration (>17 – 20 MPa) [41]. Besides, it was also found that 10% of SA is superior to 5% PC in reverse bond strength when the same duration of antioxidants treatment was performed. We suggested that it is related to the different molecular weights and the solubility of the 2 antioxidants. The molecular weight of PC is much higher than that of SA, which makes it more difficult for the PC to penetrate enamel than SA, thus achieving weak antioxidants and free radical scavenging effects. Moreover, the characteristics of PC with low solubility and high viscosity may lead to less than 5% of dissolved PC in solution. Ultimately, the adherence of undissolved PC to the surface of enamel inhibits the role played by antioxidants, thus hindering subsequent bonding operations.

Duration of antioxidant application

In the present study, the antioxidants application with 120 minutes has better MTBS values than that with 30 minutes and 60 minutes. Consequently, it is recommended that 10% of SA for 120 minutes may serve as a useful clinical alternative for patients in need of immediate esthetic rehabilitations after bleaching. Based on previous studies, the authors reported that the reparation was performed at once with 10% SA for 1 hour after bleaching, without discolor or shape change after 1 year. However, antioxidants are limited in that they gradually oxidize with time and thus become less reductive [42]. To overcome this shortcoming, 2 groups (SA120 and PC120) in this study were treated with antioxidants for 120 minutes.

Evaluation of materials bond properties

Numerous methods have been used to evaluate the bond properties of materials, including bond strength test, fracture type, SEM to observe the cohesion, micro-leakage test, and analytical chemistry [4,5,9,14,40,43–45].

Bond strength refers to the maximum load per unit area when the bond interface breaks. Each bond strength test method has its characteristics. Usually, the bond strength test, depending on the area of the bond interface, can be classified into 2 categories, namely, macro-bond strength test and micro-bond strength test. The macroscopic bond strength test (bond area >3 mm²) is prone to cohesive failure during force loading, thus affecting the outcomes of the bond strength test. In contrast, the micro-bond strength test (bond area <3 mm²) is not easy to bring about cohesion damage when compared to the macro-test, which can reflect the BS-RBE accurately [43,44]. In this study, because of the small size of the specimens, the micro-bond strength test was selected to avoid excessive cohesive stress affecting the research results.

According to previous studies, the evaluation methods of adhesive capacity include mainly Micro-shear bond strength (MSBS) test and micro-tensile bond strength (MTBS) test [36,43,44,46,47]. MSBS test has the advantages of simple specimen preparation and universal fixture. However, it is not uniform during the process of force loading, and the measured bond strength may be lower than the actual situation [43]. Meanwhile, the MTBS test has the advantages of saving tooth tissue, reducing regional differences, detecting irregular bonding interface, uniform stress distribution, and convenient observation of SEM after fracture [44]. Thus, it can better represent BS-RBE. Although the MTBS test has the shortcomings of difficult specimen making and accidental breakage of specimens, they can be prevented effectively by making specimen after embedding. Therefore, the MTBS test was utilized to access the BS-RBE in the present study.

Typically, the shape of the specimens is classified as funnel, dumbbell, and strip. Among them, funnel-shaped and dumbbell-shaped loading forces concentrate more on the bonding interface than strip-shaped loading forces, but the specimen is easy to fracture in the preparation process. Besides, it is difficult to achieve the same shape of all specimens, and irregular shape specimens are difficult to fix on the material testing machine. These factors increase the difficulty of the experiment. Therefore, a rectangular specimen with a square bonding interface was selected in this experiment. There are 3 types of fracture interface: adhesive fracture, cohesive fracture, and mixed fracture, with the type of fracture interface capable of reflecting the bond strength indirectly. The bond strength at the interface is higher for both cohesive fracture interface and mixed fracture interface. In this experiment, only cohesive fracture and mixed fracture in the unbleached-bonded group account for a high proportion, which is in line with the mechanical test results. Also, to facilitate the analysis of fracture interface types, only the enamel-resin bonding interface was studied in this experiment.

The bonding interface, as well as the morphology, density, and bonding interface of resin protrusions, can directly be observed under scanning electron microscopy [5,6,10,23,48]. In this study, adhesive type fractures accounted for 88.75%, followed by cohesive for 3.75%. Hence, we followed the recommendations proposed by Roulet et al. [49] as possible as we can in this study to prevent variability and standardize procedures.

Although the encouraging results were obtained in this study, it still involves many limitations. During the preparation process of MTBS specimens, it is difficult to control the area of bonding interface at 1 mm². In fact, the actual area of the specimens in the study was between 1 mm² and 2.5 mm², which could meet the requirements of the MTBS test. However, the results may be affected by different areas of the specimens. Besides, no micro-leakage test or aging test was conducted in this study. The micro-leakage analysis also serves as an essential parameter to evaluate the bonding interface tightness, and the aging test can determine the long-term bonding effect of bonding materials. Moreover, it is necessary to further study the combination of antioxidant treatment with other methods, such as treated with alcohol to bleached enamel, to achieve a better bonding effect.

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Conclusions

This study had a number of findings: 1) The immediate BS-RBE was decreased significantly by tooth bleaching. 2) Both 10% SA and 5% PC can significantly enhance the immediate BS-RBE. 3) 10% SA provided a comparatively more promising improvement for immediate bond strength than 5% PC when the same duration of antioxidant was applied. 4) The MTBS value increases significantly with the prolonged duration of antioxidant treatment. 5) The bond strength was restored approximately to unbleached enamel when bleached enamel was treated with 10% SA for 120 minutes. 6) As this is an *in vitro* study, conclusions should be drawn carefully, and further *in vivo* studies should be conducted to support the clinical application of this method.

Conflict of interest

None.

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