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The effect of resveratrol application on the micro-shear bond strength of adhesive to bleached enamel

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The aim is to investigate the effect of resveratrol on micro-shear bond strength (μSBS) of adhesive to enamel after 40% hydrogen peroxide application. For μSBS test, 50 teeth were obtained, 2/3 of crowns were embedded into acrylic resin. After application of hydrogen peroxide twice, teeth were randomly allocated to control group and 9 groups ($n = 15$) according to concentrations (0.5, 1, 2 μM) and application periods (10, 30, 60 min) of resveratrol. Following, composite resin was placed onto enamel surfaces using 3 tygon tubes for each tooth. μSBS test was performed and failure modes were displayed. To analyze μSBS values, Kruskal Wallis and Mann-Whitney U tests were performed. μSBS values of 1 μM resveratrol for 10 min applied group were statistically higher than control group ($p < 0.05$). 1 μM resveratrol showed higher μSBS values than 0.5 μM and 2 μM ($p < 0.05$). No significant difference was detected between application periods ($p > 0.05$). The improvement of μSBS values with 1 μM resveratrol application may be promising for clinical problems related to reduced bond strength after bleaching.

Keywords Antioxidants, Micro-shear bond strength, Hydrogen peroxide

Vital tooth bleaching is a widespread used clinical non-invasive procedure for removal stains and pigmentation on natural teeth caused by intrinsic and extrinsic factors[1]. Three fundamental vital bleaching approaches as in-office, at-home or night-guard and with over-the-counter products have been described to achieve optimal whitening[2,3]. In-office bleaching procedure which is performed at clinical supervision with adequate soft tissue protection typically utilizes high concentrations (25–40%) of hydrogen peroxide as active ingredient[4]. Hydrogen peroxide acting as a powerful oxidizing agent shows its bleaching effect on discolored teeth by diffusing into the tooth via enamel micropores and dissociating for production of unstable free radicals which will attack organic pigmented molecules leading to promote bleaching effect[4,5].

Although bleaching efficacy of in-office technique has been demonstrated by several studies[6–8], there are some concerns regarding the safety and possible side effects of hydrogen peroxide such as tooth sensitivity[9], enamel alterations[10], gingival or mucosal irritation[11], reduction in enamel micro-hardness[12], pulpal damage[13], and deteriorative effects on the structure of restorative materials promoting decreased cell viability[5,14]. In addition, previous studies[15,16] have revealed that adhesive application immediately after bleaching procedure adversely affects the bond strength which may be attributed to the morphologic alterations of enamel caused by hydrogen peroxide and the polymerization inhibition of resin adhesive systems due the presence of residual peroxide.

To accomplish the drawbacks related to compromised bond strength following bleaching procedures, the application of antioxidants such as ethanol, sodium ascorbate, acetone, catalase, alpha-tocopherol, green tea and grape seed extract have been suggested by previous studies[17–20]. Recently, the use of food-derived antioxidants against oxidative damage has drawn growing attention[21]. A polyphenolic molecule resveratrol (3,5,4′-trihydroxystilbene) which occurs naturally in peanuts, berries, grapes, and other plant sources as well as in red wine in significant amounts, has been reported to have strong antioxidant properties with anti-inflammatory, anti-proliferative, anti-aging, osteogenic and anti-cancer effects[21–23].

In a recent study[24], it has been reported that the addition of resveratrol to adhesives increases the cell viability without decreasing bond strength of adhesives. It is possible to consider that resveratrol might reduce the chair time by improving bond durability and take the initiative to longer-lasting restorations by enhancing biocompatibility of composite resin after bleaching. Longer-lasting restorations reduce the need of frequent replacements consequently which minimizes the restorative process, potentially leading to fewer intricate

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treatments and a lower risk of tooth loss. Therefore, the present study aims to investigate the effect of resveratrol application with varying concentrations and different time periods on the micro-shear bond strength of adhesives to enamel. The null hypothesis was that resveratrol would not increase the bond strength of adhesives to bleached enamel.

Materials and methods

Specimen preparation

For micro-shear bond strength test, 50 freshly extracted mandibular anterior teeth which have intact labial enamel surfaces and not exposed to chemical pre-treatment were obtained. The protocol was approved by Institution Committee for Ethics of Research. The extracted teeth were kept in 0.5% Chloramine-T solution at 4 °C for a duration not exceeding 1 week after extraction. Then, the teeth were kept in 37 °C distilled water until use. The teeth were examined in terms of defects like caries or fractures, and those with defects were excluded. After cleaning soft and hard deposits on the teeth, a high speed water-cooled diamond bur was used for separation of the roots from the cemento-enamel junction. The crowns were examined again and 2/3 of the crowns were embedded into auto-polymerizing acrylic resin (SC, Imicryl Dental, Türkiye) in a cylindrical shaped mold where the labial surfaces were at upper side. After acrylic resin was set and an exposed labial enamel surface was obtained, the enamel surfaces were grounded using 600, 800 and 1200 grid abrasive papers respectively to obtain a uniform surface. Finally, the specimens were washed and immersed in 37 °C distilled water for 24 h.

Bleaching application

As in-office bleaching agent, 40% hydrogen peroxide (Opalescence Boost, Ultradent Products, South Jordan, UT, USA) was applied. Following immersion period in distilled water, the tooth samples were dehumidified with a blotting paper and air flow. Then, Opalescence Boost was placed with a thickness of 1 mm onto the labial surfaces of each tooth for 20 min and at every 5 min the gel was activated with a micro-brush by following the manufacturer's instructions. At the end of 20 min, a high pressure water flow was used to wash the teeth and then the teeth were blotted with a paper and air flow. This process was re-applied with an interval of 2 days. Until the second session of bleaching, all the specimens were kept in 37 °C distilled water.

Resveratrol application

In the process of obtaining a stock solution of resveratrol (R5010 500 MG, Sigma-Aldrich, St Louis, MO, USA), it was decomposed in ethanol (50 mg/mL). To obtain adequate study concentrations, the stock solution of resveratrol was diluted in physiologic serum[23]. After bleaching, the tooth specimens were randomly divided into 10 groups according to the concentrations (0.5µM, 1µM, 2µM) and time periods (10, 30, 60 min) of resveratrol application. One group serves as a control group without resveratrol application.

For micro-shear bond strength (µSBS) test, before the placement of cylindrical shaped tygon tubes, enamel surfaces were etched with 35% phosphoric acid (Ultra-Etch, Ultradent Product, South Jordan, UT, USA) for 15 s. After rinsing and blot drying, resveratrol was applied to the specimens with a micropipette and treated with a micro-brush[25,26]. Following gentle air drying, Scotchbond Universal Adhesive (SU, 3M Oral Care, St Paul, MN, USA) was applied to enamel surfaces with a micro-brush as 1 layer and rubbed for 20 s. Then the adhesive was gentle air-dried for 5 s to evaporate the solvent. The adhesive protocol was implemented following the manufacturer's guidelines. Onto each tooth sample, 3 tygon tubes with a 1 mm thickness and 0.75 mm diameter were positioned, then adhesive was light-cured for 20 s in standard mode using a LED device (Planmeca Lumion, Planmeca Oy, Helsinki, Finland) with a 1000 mW/cm^2 light intensity. Afterwards, the tubes on the specimens were filled with a nanohybrid composite resin (Clearfil Majesty Esthetic, Kuraray Noritake Dental Inc.; Okayama, Japan) and light-cured for 20 s. After immersion of the samples in distilled water at 37 °C for 24 h, a sharp scalpel was gently used to cut and remove the tygon tubes. Eventually, a total of 150 composite resin cylinders ($n = 15$) were obtained. The number of specimens which will be examined was determined via a power analysis using G*Power, with a power of 90% and an assumed significance level of 0.05.

Micro-shear bond strength test

A universal testing machine (EZtest-500 N Shimadzu, Kyoto, Japan) was used to conduct micro-shear bond strength test. A 0.2-mm-diameter wire was positioned around the composite resin cylinder as half of the resin base was in contact with the labial surface of the crowns[27]. A shear force with a 1.0 mm/min crosshead was loaded to each specimen until debonding was occurred. After the µSBS values at failure were obtained, the conversion of Newton's to MPa was achieved by dividing maximum load causing debonding to the surface area of the composite resin cylinder (mm^2) (Fig. 1).

Failure modes of specimens were microscopically observed by 1 operator at a 40X magnification (Olympus SZ61, Olympus Optical, Tokyo, Japan) to determine the fracture patterns which were classified as adhesive failure between the enamel and composite resin, cohesive failure within the composite resin and the mixture of adhesive and cohesive failures.

Statistical analysis

Shapiro-Wilk normality test was used for descriptive statistics and to control the distributions for each group. For the analysis of µSBS values among 10 groups, Kruskal Wallis test was performed. Mann-Whitney U test was used for pairwise comparisons. To reveal the differences and effects of parameters according to the application period and concentration of resveratrol, two-way ANOVA was performed. $p < 0.05$ (Bonferroni adjusted alpha = 0.05) was accepted as statistically significant.

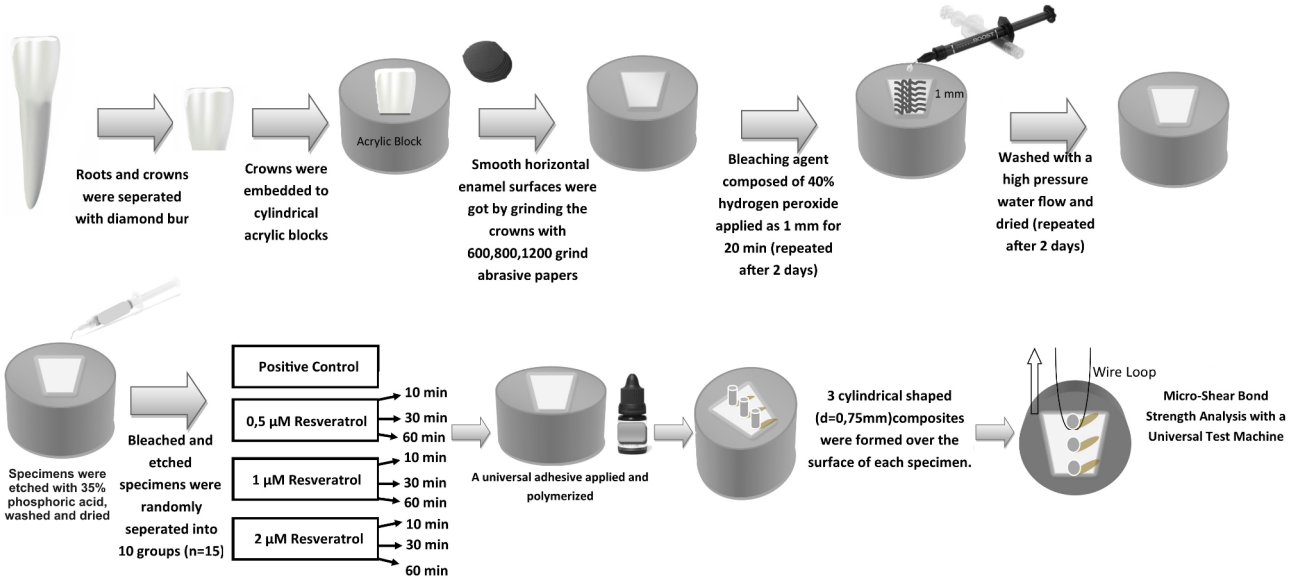

Fig. 1. Schematic representation of micro-shear bond strength test.

Groups	µSBS values (MPa) (Standard deviation)
Control group	15.47 (2.53)
0.5 µM 10 min	17.09 (1.20)
0.5 µM 30 min	12.03 (3.64)
0.5 µM 60 min	9.54 (2.12)
1 µM 10 min	28.02 (1.57)
1 µM 30 min	19.45 (2.02)
1 µM 60 min	21.78 (2.19)
2 µM 10 min	14.01 (1.04)
2 µM 30 min	18.77 (3.62)
2 µM 60 min	13.03 (2.28)

Table 1. The mean µSBS values (MPa) (standard deviation) of control and resveratrol applied groups with different concentrations and time periods.

Results

The mean µSBS values and standard deviations of the groups are presented in Table 1. *p* values and statistical differences obtained by the multiple comparisons of the groups are presented in Table 2; Fig. 2. Regarding µSBS values, µSBS values of 1 µM 10 min group were statistically higher than control group ($p < 0.05$). Regardless of the application period, 1 µM applied specimens showed significantly higher µSBS values than the concentrations of 0.5 µM and 2 µM ($p < 0.05$). There was no statistically significant difference among the application periods regardless of the concentration ($p > 0.05$). According to the multiple comparisons, 1 µM 10 min group exhibited the highest µSBS values with statistical difference between control group, 0.5 µM 30 min, 0.5 µM 60 min, 2 µm 10 min and 2 µm 60 min groups. The lowest µSBS values were observed in 0.5 µM 60 min group which were statistically lower than 0.5 µM 10 min, 1 µM 10, 30, 60 min and 2 µM 30 min groups. When the effects of parameters included in this study were analyzed, it was found that only concentration had an effect on the results ($p < 0.001$) while R2 value was 0.284.

The number of failure modes of the groups are shown in Fig. 3. According to the microscopic evaluation, the most observed fracture pattern was adhesive failure. While control group, 0.5 µM 10, 30, 60 min groups, 2 µm 10 min and 60 min groups shows mostly adhesive failure; for 1 µm 30 and 60 min groups; the majority of the failures was mixed failure.

Discussion

For the maintenance and success of the composite resin restorations, the quality and durability of adhesion formed between adhesive systems and the enamel is of great importance[17]. For reversal of the compromised bond strength of adhesives to enamel after bleaching, the most common recommendation was to delay any bonding procedure following bleaching applications for variable periods of 24 h to 3 weeks[17,28]. The application of antioxidant agents was first suggested in a previous study by Lai et al.[29] which mentioned that compromised

p values	Control group	0.5 µm 10 min	0.5 µm 30 min	0.5 µm 60 min	1 µm 10 min	1 µm 30 min	1 µm 60 min	2 µm 10 min	2 µm 30 min	2 µm 60 min
Control group		0.894	0.247	0.092	0.004*	0.721	0.351	0.400	0.690	0.260
0.5 µm 10 min	0.894		0.287	0.019*	0.050	0.421	0.286	0.625	0.564	0.098
0.5 µm 30 min	0.247	0.287		0.564	0.007*	0.061	0.092	0.657	0.108	0.858
0.5 µm 60 min	0.092	0.019*	0.564		0.001*	0.004*	0.003*	0.030*	0.030*	0.855
1 µm 10 min	0.004*	0.050	0.007*	0.001*		0.118	0.328	0.004*	0.197	0.004*
1 µm 30 min	0.721	0.421	0.061	0.004*	0.118		0.592	0.073	0.964	0.031*
1 µm 60 min	0.351	0.286	0.092	0.003*	0.328	0.592		0.142	0.689	0.028*
2 µm 10 min	0.400	0.625	0.657	0.030*	0.004*	0.073	0.142		0.156	0.140
2 µm 30 min	0.690	0.564	0.108	0.030*	0.197	0.964	0.689	0.156		0.980
2 µm 60 min	0.260	0.098	0.858	0.855	0.004*	0.031*	0.028*	0.140	0.980	

Table 2. *P* values and statistical differences of the control and resveratrol applied groups according to the multiple comparisons. *Statistically significant difference ($p < 0.05$).

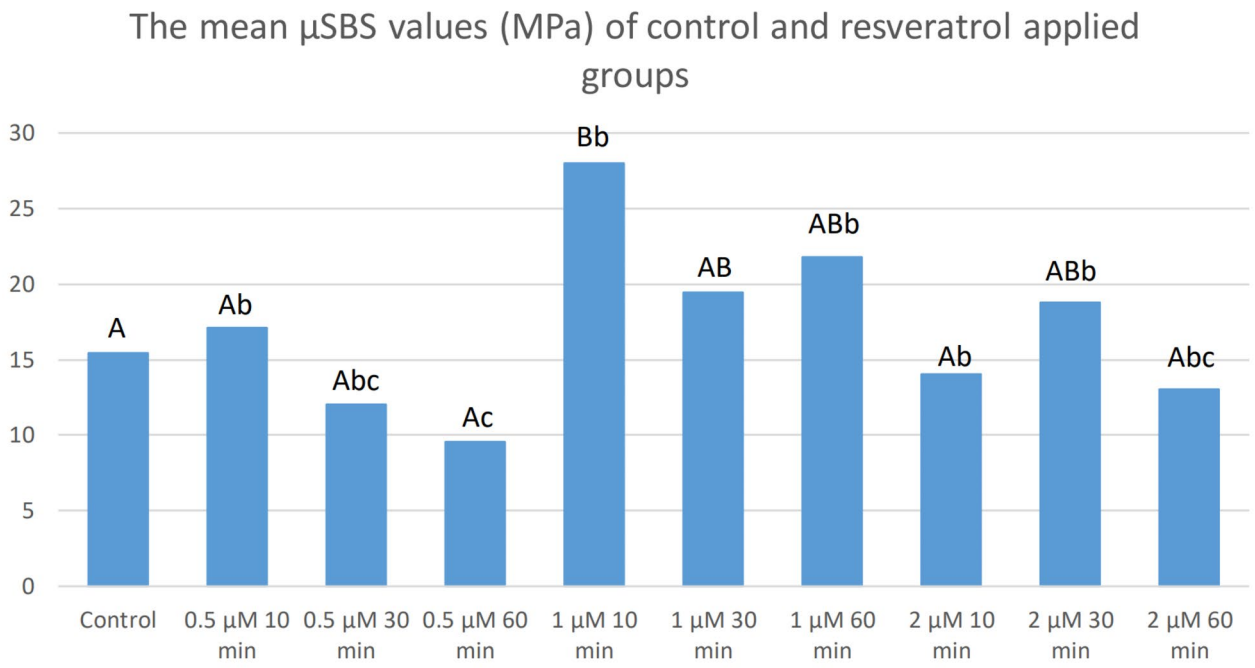

Fig. 2. The mean µSBS values (MPa) of control and resveratrol applied groups. Different letters indicate statistically significant differences ($p < 0.05$).

bond strength observed after bleaching can be reversed with sodium ascorbate treatment. This result was supported by several studies[17–19,30] and the application of other antioxidant agents has been recommended[19,20,31]. Recently, the utilization of natural plant extracts and food-derived antioxidants as a viable alternative to chemical and synthetic antioxidants have been encouraging[32]. Sharafeddin et al.[33] reported that 5% and 10% grape seed extract, 5% and 10% pomegranate peel extract, 5% green tea extract and aloe vera leaf gel may be an alternative to 10% sodium ascorbate to reverse the compromised bond strength of adhesives to bleached enamel. However, these extracts have disadvantageous properties as containing pigments which may interfere with the bleaching results[34], challenges in solution preparation and the potential impact of the preparation method on test results[35]. As an alternative antioxidant, the effect of resveratrol, which is a naturally occurring polyphenolic compound

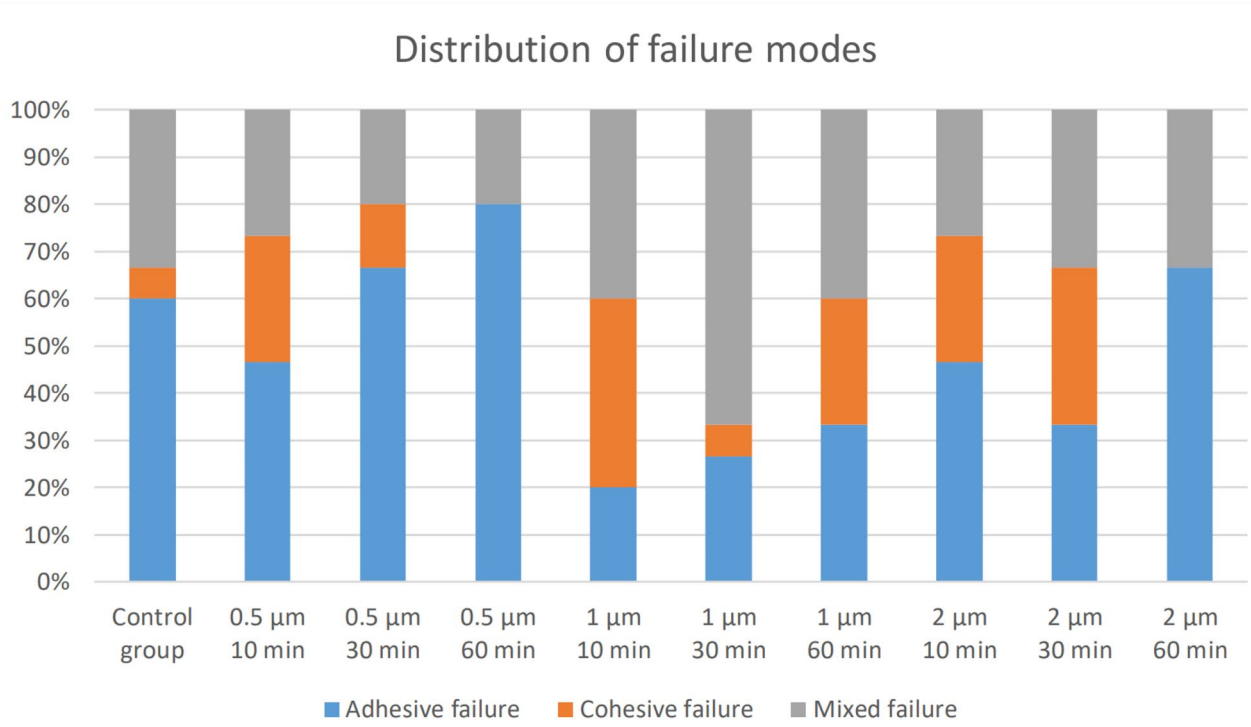

Fig. 3. Distribution of failure modes for control and resveratrol applied groups.

with powerful antioxidant activity, on the micro-shear bond strength of adhesives to bleached enamel was evaluated in this study.

In recent years, resveratrol has become a bioactive option for incorporation in dental adhesives to enhance bond durability and antibacterial capability[25]. Beyond its strong antioxidant functions, resveratrol has been reported to decrease the acid production rates of *S. mutans* at sub-MIC concentrations by inhibiting the bacterial glycolytic pathway[36], suppress the expression of MMP-2 and MMP-9 in various tissues or cells[37], preserve the collagen fibrils within the hybrid layer[38], reduce nanoleakage[25], and promote biomimetic remineralization[39]. These functions may lead resveratrol to be in a more advantageous position compared to other antioxidants. Therefore, resveratrol was chosen as an antioxidant for the present study.

It has been reported that resveratrol ensures diverse health benefits in a dose-response manner[40]. Atalayın et al.[41] evaluated 9 different concentrations (0.25–100 µM) of resveratrol in terms of cell viability and the most protective dose of resveratrol that significantly enhance the cell viability was reported to be 0.5 µM. Guo et al.[25] reported that the bond strength of adhesives could be negatively affected by using excessive concentration of resveratrol. Taking these reports into consideration, 3 different concentrations (0.5 µM, 1 µM, 2 µM) of resveratrol that do not cause cell damage were applied to bleached enamel in the present study. According to the µSBS results, regardless from the application period, 1 µM applied specimens showed significantly higher µSBS values compared to the concentrations of 0.5 µM and 2 µM. Consistent with the results of the present study, Brock et al.[34] reported that 10% resveratrol was able to restore the bond strength to bleached enamel in a short application time of 5 min. These results have clinical significance since concentrations and applications periods which was demonstrated as safe and effective may be adopted in future experiments regarding the bond strength of adhesives.

It has been mentioned that the reversal effect of antioxidant agents in bond strength of adhesives to enamel following bleaching procedures depends on concentration and also application time of antioxidant agents[30,42]. Türkün et al.[17] reported that the application of sodium ascorbate for 10 min is an adequate period to reverse the decreased bond strength, however, Dabas et al.[43] showed that 60 min antioxidant application resulted an increase in bond strength values and suggested that bond strengths of composite resin to bleached enamel increased with an increase in the application time of sodium ascorbate. In the present study, resveratrol was applied to bleached enamel in 3 different time periods (10, 30, 60 min) and no statistically significant difference among the application periods was observed. Not consistent with this result, in a previous study[34] which has evaluated 5 time periods (5, 10, 15, 30, 60 min) of resveratrol application to the bleached enamel, it was found that higher bond strength values were obtained after 5 and 10 min when compared to 15, 30 and 60 min resveratrol application periods. The discrepancy between the results may be attributed to the differences in bonding protocol and varying concentrations of resveratrol.

Resveratrol shows its antioxidant effect by a variety of antioxidative mechanisms, such as inhibiting production of reactive oxygen species, scavenging free radicals, and stimulating biosynthesis of endogenous antioxidants[38] which is similar to sodium ascorbate and other antioxidant agents. As reported by a previous

study[38], resveratrol obtains its anti-inflammatory and antioxidant properties primarily from the existence of phenolic hydroxyl groups. Studies have documented that natural polyphenolic extracts like proanthocyanidin and epigallocatechin-3-gallate can induce collagen crosslinking effects through hydrogen bonding between phenolic hydroxyl groups and protein amide carbonyl[25,26]. In this manner, resveratrol was expected to increase the reduced bond strength of adhesives to bleached enamel by these mechanisms. According to the results of the present study, 10 min applied 1 μM resveratrol group showed statistically higher μSBS values. With that result, the null hypothesis of the present study was rejected. As consistent with the results of the present study, Guo et al.[25] indicated that the incorporation of 0.1 and 1 mg/mL resveratrol to the adhesive improved dentin bond strength after thermocycling. The lack of thermocycling procedure is a limitation of the present study.

According to Peng at al[26] as the concentration of resveratrol/ethanol pretreatment increased, there was a notable enhancement in microtensile bond strength to dentin following thermocycling and collagenase aging. Additionally, there was a substantial decrease in fractures occurring on the dentin side. In that study, it was speculated that resveratrol creates some biological modification on collagen fibers, therefore it can maintain or even increase the strength of collagen fibers in order to protect the integrity of the hybrid layer which may be related to the improved bond strength. However, these results are not in line with the results of the present study since 1 μM applied specimens presented higher μSBS values than the concentrations of 2 μM. The fact that the evaluated tissue in the previous study was dentin, and dentin has a higher collagen fibril content compared to enamel, could be a possible reason for the difference in results considering the effect of resveratrol on collagen fibrils.

In the present study, an ethanol-based adhesive system was selected due to the advantage of interaction with residual oxygen which may minimize the adverse effect of bleaching on the bond strength of adhesive[34]. Regarding the etching procedure, different protocols have been observed. While Guo et al.[25] and Peng et al.[26] applied the acid before the resveratrol application, Brock et al.[34] etched the enamel surface after the application of resveratrol. To obtain a favorable hydrophobicity and a proper interaction between resveratrol and ethanol, resveratrol was applied following the etching procedure in the present study.

The antioxidants may be applied to enamel in solution and gel forms[43]. Kimyai et al.[44] speculated that 10% sodium ascorbate hydrogel might not be effective as 10% sodium ascorbate solution, since the additives within the hydrogel may limit the efficacy of the material. On the other hand, Türkün et al.[45] showed that 10% hydrogel form of sodium ascorbate was as effective as the solution form, but lower concentrations of hydrogel did not have a significant effect on improving the bond strength after bleaching. In the present study, the solution form of resveratrol was applied to enamel to determine the effective dose. In a previous study[43], it has been reported that hydrogel form is easy to apply due to its higher viscosity and better control than solution form and needs shorter chair time. Therefore, the hydrogel form of resveratrol which may be more acceptable for clinical utilization should also be evaluated.

Regarding the failure modes of control and resveratrol applied groups; the majority of adhesive failures observed in 0.5 μM and 2 μM resveratrol applied groups revealed that a strong bond was not formed in these groups which may be attributed to the concentration of resveratrol. For all groups, the small bonded cross-sectional areas (1 mm^2 or less) required for the micro-shear bond strength test may be the reason of adhesive failures.

In addition of all its advantages, as reported by Peng et al.[26], resveratrol may have a potential cytotoxic effect which depends on the concentration and the negative effects of polyphenols as radical scavengers may affect the polymerization of composite resin[25], therefore optimal attention is needed for the use in clinical applications.

Conclusions

On the basis of the results of this study and within the limitations of an in-vitro evaluation, it may be concluded that application of 1 μM resveratrol for 10 min improves the bond strength values of adhesives to enamel after bleaching. The reversal effect of resveratrol on the bond strength of adhesives to bleached enamel depends only on concentration, not application periods.

Further studies that evaluate the effect of different concentrations, application periods and different forms of resveratrol on biocompatibility and the bond strength of adhesives to bleached enamel are needed.

Data availability

Data is provided within the manuscript.

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Declarations

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